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# ERRATA AND AUTHORS' EMENDATIONS

Page 455, sixth line from bottom, insert after "second" the words "kernel of the."
Page 510, paragraph 3, line 4, change "its" to "their."
Page 524, line 3, $\frac{d\phi}{d_1}\varrho$ should be $\frac{d\phi}{di_1}\varrho$; line 5, $\frac{d\rho}{dA}$ should be $\frac{d\phi}{dA}$; line 6, $\frac{d\phi}{dK}-(t-t_1)\,A\,V$ should be $\frac{d\phi}{dK}=(t-t_1)\,A\,V$; line 8, $V=\frac{2.302585.10^{K(t-t_1)}}{[1+10^{K(t-t_1)}]^2}$ should be $V=\frac{2.302585.10^{K(t-t_1)}}{[1+10^{K(t-t_1)}]^2}$; line 10, $\frac{d\rho}{dK}$ should be $\frac{d\phi}{Kd}$; line 18, "are given in Tables 5 and 6," should be "are given in Tables 3 and 4."
Page 549, line 10, "$A_5$" should be "$A_6$"; line 13, "R" should be "K."
Page 576, ninth line from bottom, insert word "strictly" between "not" and "legitimate."
Page 651, line 1, the exponent "9" should be "4."
Page 770, last sentence in paragraph 3 should be at the end of paragraph 1.
Page 774, third line from bottom should precede fourth line from bottom.
Page 999, third line from bottom should read ". . . northwest, southwest, and southeast . . . ."
Page 1021, insert "21" between "18" and "24" in the heading of Table 3.

JOURNAL OF AGRICULTURAL RESEARCH

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# PRODUCTION OF FERTILE HYBRIDS IN THE ASCOMYCETE NEUROSPORA[1]

By B. O. DODGE

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## INTRODUCTION

The fact that monosporous mycelia of certain species of fungi in each of the large groups produce "perfect stages" only when mated with their reciprocal haplonts is now well established, and this type of segregation is referred to as heterothallism. In recent years various attempts have been made to produce hybrids by crossing different races, varieties, and species of fungi. Saito and Naganiski (*8*) [2] report the formation of zygospores in crosses of different forms of the Mucoraceae. Vandendries (*10*) obtained clamp connections in cultures where a strain of *Panaeolus campanulatus* was grown with a strain of *P. fimicola*. No fruit bodies were formed. Zattler (*12*) crossed two varieties of *Schizophyllum commune* and also two varieties of *Collybia velutipes*. The results, the author thinks, are proof of sexuality in the forms crossed. Burgeff (*3*) obtained zygospores when he crossed *Phycomyces nitens* with *P. blakesleeanus*, but the zygospores did not develop mycelia. Kniep (*7*) has secured conjugations between conidia, and more recently Dickinson (*4*) and Bauch (*2*) report fusions between germ tubes from sporidia of different species or races of smuts. These results are all highly interesting and valuable contributions to the genetics of fungi. The development of hybrid zygospores such as have been reported, or fusions between conidia or between hyphae of species of these groups of fungi, have not, it seems, so far resulted in the production of haplont spores or mycelia of a new generation.

In reporting on the culture work done in connection with their studies on heterothallism and homothallism in the genus Neurospora, Shear and Dodge (*9*) state that ascocarps were also secured by growing reciprocal haplonts of *N. sitophila* and *N. crassa* together, and by similarly mating *N. tetrasperma* with each of these two species. The manner of development of the fruit bodies and the characters of the ascocarps and their ascospores were such as to suggest that the ascocarps were probably true hybrid structures. The writer has continued the culture work with the three different hybrids. The results so far obtained by crossing the two eight-spored species, *N.*

[1] Received for publication Dec. 1, 1927; issued February, 1928.

[2] Reference is made by number (italic) to Literature cited, p. 14.

*sitophila* and *N. crassa*, with the four-spored species, *N. tetrasperma*, illustrate very strikingly some of the principles involved in hybridizing ascomycetes.

## MATERIALS

### MORPHOLOGICAL FEATURES OF SPECIES CROSSED

Species of Neurospora furnish excellent material for genetical studies because of the ease with which true hybrid perithecia can be produced. The ascospores of these hybrids can be made to germinate and the mycelia in turn be made to give new generations of ascospores. The writer is referring here to hybrids between distinct species and not to crosses between mere races or varieties of the same species. The original descriptions (*9*) of the three species of Neurospora bring out the diagnostic features. *N. crassa* and *N. sitophila* are most nearly alike in their conidial condition. The ascospores are, however, very readily distinguished, and their ascocarps are unlike in size, color, and other characteristics. The perithecia of the former are larger and more nearly black than are those of the latter. These two species have been crossed and the ascospores from the fruit bodies which matured have been germinated. About 60 haplont mycelia so obtained have been grown in various combinations, with some highly interesting results. It is desired to report here, however, principally on the work done with *N. sitophila* and *N. tetrasperma* because of the more striking specific differences between these two species.

### CYTOLOGICAL FEATURES

The writer has recently (*5*) described nuclear behavior in the ascus of *Neurospora tetrasperma*, showing that each of the four spores originally includes two nuclei of opposite "sex." Furthermore, any spore which contains only one nucleus at the time it is cut out is much smaller than are normal spores, and it is unisexual. A haplont mycelium from such a small spore must be mated in culture with one of reciprocal sexuality in order to obtain perithecia. In other words, the normal spores of this species are homothallic or bisexual, and the small spores are unisexual or heterothallic. In the paper referred to above it was shown that segregation of the sex factors could take place in any one of the three nuclear divisions in the ascus and still result in the formation of bisexual spores. It seems probable, however, that segregation normally takes place in the first division. Wilcox (*11*) has determined culturally that in *N. sitophila* the spores in each half of an ascus alternate in pairs, two of one sex and two of another. Interpreting the results of her culture work in the light of what is found regarding nuclear behavior in the ascus, it is pointed out that here segregation of the sex factors must regularly take place in the second division of the ascus.

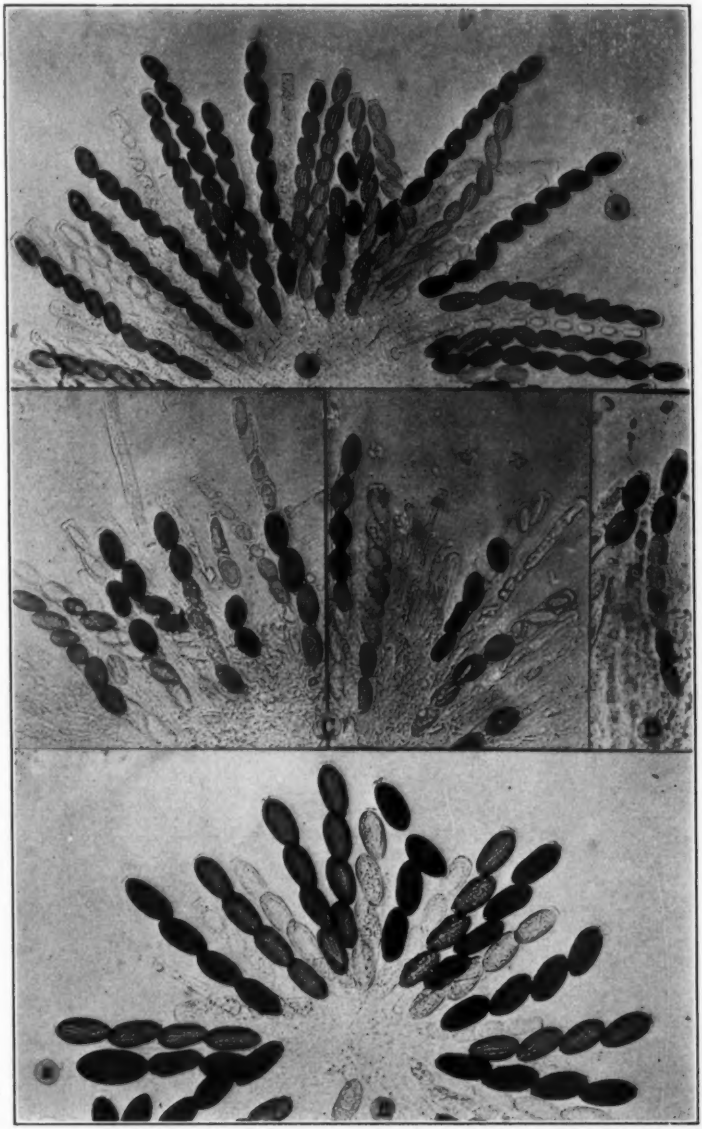
---

EXPLANATORY LEGEND FOR PLATE 1

A.—*Neurospora sitophila;* $p_1$ ascospores, Arlington strain. Asci regularly 8 spored. Only one ascus, *a*, with mature spores. The younger spores show the anastomosing striations more distinctly. ×315

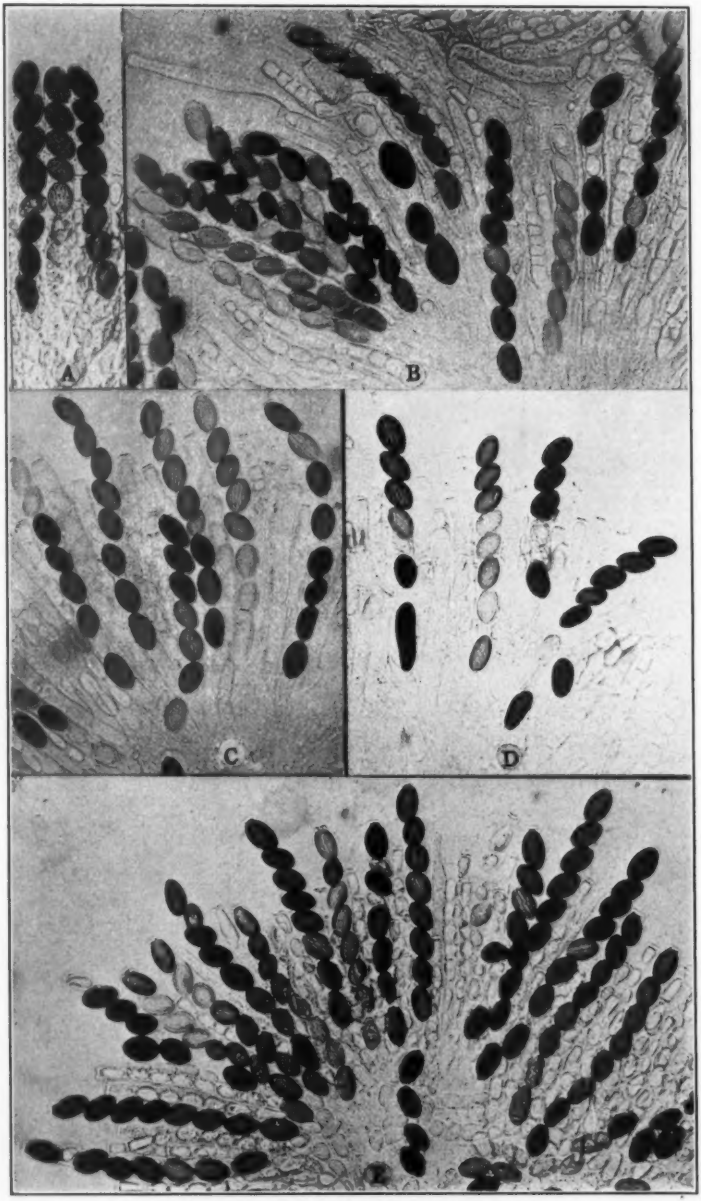
B.—*Neurospora tetrasperma;* $p_2$ ascospores. All asci 4 spored except one, *a*, which has 1 large spore and 2 of normal size. Anastomosing ridges on the spores not as distinct as they are in the preceding species. ×315

C and D.—First-generation ($f_1$) hybrid ascospores. Asci from a hybrid perithecium obtained by crossing *Neurospora sitophila* and *N. tetrasperma*. All asci shown here have 8 spores; many spores aborted after delimitation. Masses of mucilaginous material and partly disorganized hyphae among the asci mature ascospores varying somewhat in size. Rarely only 6 or 7 ascospores are delimited in such hybrid perithecia. ×315



Asci of Neurospora sitophila, A; N. tetrasperma, B; and sitophila × tetrasperma, C, D.

For explanatory legend see page 2




Asci from second and third generations

For explanatory legend see page 3

The cytology of the cross *Neurospora tetrasperma* × *N. sitophila*, and particularly nuclear behavior in the asci in perithecia resulting from back crosses, is also proving highly interesting. The results so far obtained go to show most emphatically the necessity for cytological study as a basis for a correct interpretation of the results of culture work.

### COMPARISON OF CHARACTERISTICS OF SPECIES CROSSED

Some of the characteristics of the two species of Neurospora crossed are given below in parallel and perhaps in order of their importance and reliability as marks of identification. Certain of these features are brought out in the works referred to (*5*, *9*, *11*) and in the illustrations included in this paper. Plate 1 shows asci and spores of *Neurospora sitophila*, *N. tetrasperma*, and of the first-generation cross. Plates 2, 3, and 4 show asci and spores of different generations of hybrids.

| *Neurospora sitophila* | *Neurospora tetrasperma* |
|---|---|
| 1. Heterothallic. | 1. Homothallic. |
| 2. Asci eight spored. | 2. Asci four spored. |
| 3. Segregation of sex factors in the second division. | 3. Segregation of sex factors probably in first division. |
| 4. Ascospores at first uninucleate, at maturity binucleate. | 4. Ascospores at first binucleate, at maturity four nucleate. |
| 5. Ascocarps begin to discharge spores within two or three weeks; fully mature in about three or four weeks. | 5. Ascocarps begin to discharge spores in about 10 days; fully mature in two weeks. |
| 6. Ascospores mostly 20–25 μ long. | 6. Ascospores mostly 30–33 μ long. |
| 7. Ascospores rather strongly ribbed. | 7. Ascospores faintly ribbed. |
| 8. Conidia bright salmon pink, profuse on corn-meal agar. | 8. Conidia pale salmon pink to whitish, very sparse on corn-meal agar. |
| 9. Perithecia 250–350 μ in diameter. | 9. Perithecia 200–275 μ in diameter. |
| 10. Asci long and narrow. | 10. Asci rather short. |

There is of course some variability in the features noted above. For example, when an ascus of *Neurospora tetrasperma* matures five or six spores, the spores will be of two different sizes, and the smallest spores will then be uninucleate and unisexual (*5*). Mycelia from these small spores develop scarcely any conidia on corn-meal agar. As regards the size of the perithecia, there is likewise some overlapping in the measurements in particular cases, and for each species the fruit bodies vary in size somewhat according to environmental conditions and according to the number produced in the culture. More numerous measurements show that the dimensions given by Shear and Dodge (*9*) do not indicate the differences in size accurately, but the figures in their Plate 4, A and B, show that the perithecium of

### EXPLANATORY LEGEND FOR PLATE 2

A, B, and C.—Second-generation ($f_2$) hybrid ascospores; asci from perithecia obtained by mating mycelia Nos. 8 and 9, derived from $f_1$ ascospores (pl. 1, C); perithecium developed as the result of crossing *Neurospora sitophila* and *N. tetrasperma*. Note some unevenness in maturity of the spores. Asci all 8 spored except the one shown at the center in B. This ascus originally delimited 5 spores, only 3 of which are approaching maturity; the 2 in the upper end are still hyaline. Counting from above, the 8 original nuclei are probably distributed as follows: 2, 1, 2, 1, 2 × 315

D.—Third-generation ($f_3$) hybrid ascospores; asci from a perithecium obtained by mating Nos. 273 and 274, derived from $f_2$ ascospores similar in origin to those shown in A, B, and C. One ascus, at the left, delimited only 7 spores, one of which is much larger and probably contained 2 nuclei at its origin. × 315

E.—Third-generation ($f_3$) hybrid ascospores; asci from a perithecium obtained by mating $f_2$ mycelia Nos. 256 and 233 derived from spores of an origin similar to Nos. 273 and 274. All asci 8 spored. Morphologically such perithecia would not be distinguished from perithecia of *Neurospora sitophila*. × 315

*N. tetrasperma* is only about three-fourths to four-fifths the size of the perithecium of *N. sitophila*. Measurements of 50 perithecia of each species at about the same stage of maturity indicate that this is about the average ratio of their sizes. With an abundance of material for study one usually has no difficulty in distinguishing the two species even if he considers only one of the diagnostic features noted above.

## METHODS

The method of inducing certain types of ascospores to germinate by heating them to 60 or 70° C. or more for a few minutes was originally worked out by the writer in connection with his studies on the Ascobolaceae. Additional special methods for culturing the Neurospora forms have also been rather fully described (*9*) and the precautions which are so necessary in this work have been emphasized. In case one is to work with cultures to be derived from ascospores from hybrid perithecia, the importance of excluding conidia is apparent. When haplont A of one species is grown with a reciprocal haplont B of the other parent species, and perithecia mature, the culture tube contains conidia of both parents in addition to ascospores from the hybrid fruit bodies.

The process by which the homothallic species *Neurospora tetrasperma* can be crossed with the heterothallic species *N. sitophila* will be described again briefly. In any spore print of the former species one can find a few small spores which measure from 20 to 25 $\mu$ or thereabout in length. As a rule, such small spores of this species are unisexual, as already noted. Of a number of small spores measured and later proved to be unisexual, the smallest was 18 by 11 $\mu$ and the largest was 29 by 12.5 $\mu$. The average of 23 spores was 24.3 by 11 $\mu$. The average size of the bisexual spores is about 31 by 14–15 $\mu$. By growing the mycelia derived from the unisexual spores in various combinations, one can readily determine which are haplonts A and which haplonts B (*9*).

When any haplont A of *Neurospora tetrasperma*, for example, is grown with a haplont B of *N. sitophila* on corn-meal agar, there occurs a reaction which results at about the end of one week in the development of perithecial primordia scattered over the surface of the agar slant. While only a very few of these structures finally

EXPLANATORY LEGEND FOR PLATE 3

A.—First-generation back-cross ascospores; perithecium obtained by mating the mycelium $S_6$ (haplont A) of the parent species *Neurospora tetrasperma* with the first-generation ($f_1$) hybrid mycelium No. 5, derived from an ascospore from the cross *N. sitophila* × *N. tetrasperma*. Asci with variable numbers of spores, from 3 to 8. No abortion of spores, the small spores containing only one nucleus at their origin and the larger spores more than one. × 180

B.—Spores from a spore print of another back cross of similar origin except that $S_1$ (haplont B) was mated with the $f_1$ mycelium No. 1. The largest spore shown is 57 $\mu$ long; the shortest is only 18 $\mu$ long. Such spores germinate readily when properly heated. × 315

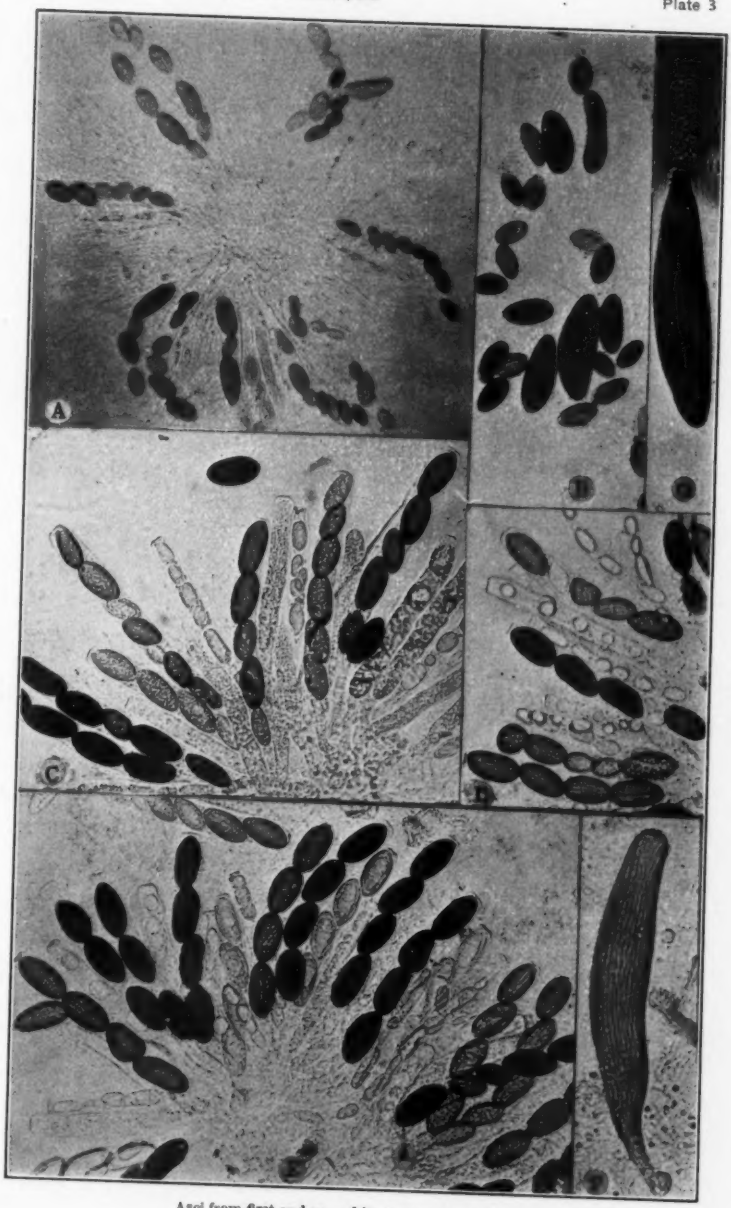
C.—Second-generation back-cross ascospores. Asci with from 4 to 7 spores. Perithecia obtained by mating mycelium $S_1$, a haplont B of *Neurospora tetrasperma*, with mycelium No. 207, derived from a small ascospore of an origin similar to those shown in A. Most of the asci from this perithecium have more than 4 spores. × 315

D.—Another second-generation back cross resulting from similar mating, $S_1$ × 208. Some asci in these perithecia have 4 spores, others have 5. There is some irregularity as to shape and size in the 5-spored type. × 315

E.—Asci from another perithecium matured in the same culture as those shown in D. All asci here are 4 spored except two 5-spored asci shown at *a*. × 315

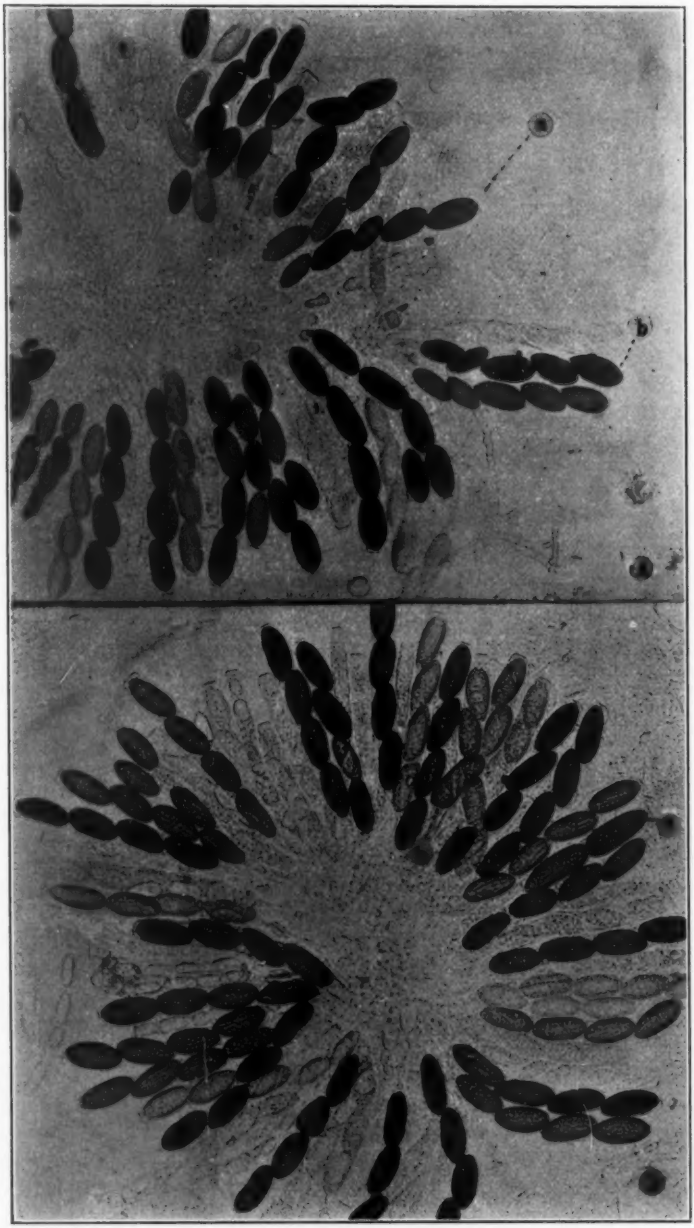
F.—Olive-brown indurated ascus showing anastomosing ridges similar to those usually found on the walls of ascospores; no spores develop in such asci. The cause for the formation of these aborted asci has not been determined. Compare with G, a single-spored ascus. × 570

G.—One-spored ascus of a back-cross hybrid. The end of the ascus shows above, the lower end not visible in the illustration. Compare with F. × 570




Asci from first and second back-cross generations

For explanatory legend see page 4




Asci of Neurospora tetrasperma, A, and of a second generation back cross, B

For explanatory legend see page 5

mature ascospores, they are young perithecia and essentially different from the sclerotiumlike bodies which so often develop in vast numbers in certain monosporous cultures.

As soon as small brown or blackish bodies begin to appear one has no difficulty in distinguishing which cultures contain reciprocal haplonts. These hybrid perithecia frequently fail to mature the ostiolar structure, but when a short beak with an ostiole is finally formed it may be taken to indicate that at least a few ascospores will eventually mature. It is on account of the slowness with which these structures mature that the writer is not as yet ready to furnish an adequate description of the first-generation perithecia. The first essential seemed to be the accumulation of mature $f_1$ ascospores for further work.

## DESIGNATION OF MYCELIA AND HYBRID STRUCTURES

Mycelia of species of Neurospora produce coiled structures which, very likely, are morphologically equivalent to sex organs. The writer has made no attempt as yet to determine which haplonts are male and which are female. Sexual reproduction in these forms is a problem in itself which should receive careful study before definite statements are made in regard to the sex of particular haplonts. The two mycelia which are opposite in their reaction in the production of perithecia have been classed provisionally as haplonts A and B (*9*). Where symbols are helpful in the discussion, the system adopted by Allen (*1, p. 558*) may be used. The ancestral parents, *Neurospora sitophila* and *N. tetrasperma*, will be represented by $p_1$ and $p_2$. The Columbia University strain of the former species being a haplont A, and the $S_1$ strain of the latter species a haplont B, the first cross would be represented by $p_1A \times p_2B$. The $F_1$ diploid generation consists of ascogenous hyphae and the young uninucleate asci. The $f_1$ generation, begun with the completion of reduction divisions in the ascus, is represented by ascospores, their mycelia and conidia. Where a first-generation hybrid mycelium is mated with a haplont mycelium of the original parent, it is referred to in the usual way as a back cross and the resulting ascospores as first-generation back-cross spores, second-generation back-cross spores, etc., or, perhaps better, simply as spores from the first back cross, second back cross, etc.

## FIRST-GENERATION HYBRID STRUCTURES

### PERITHECIA

The hybrid perithecia are essentially like those of the *sitophila* parent, averaging about the same size or a little larger. At the end of about one month the first ascospores can be found. Crushed mounts show a great irregularity in the size and state of maturity of spores in an ascus. As the perithecia are allowed to grow further it is clear that many asci contain eight spores, some hyaline and

EXPLANATORY LEGEND FOR PLATE 4

A.—Asci from a perithecium of the $p_2$ parent, *Neurospora tetrasperma*. At *a* and *b* are shown 3 asci which have 5 spores each; at *c* an ascus with only 3 spores. Compare with asci containing second-generation back-cross ascospores, shown in B. × 315

B.—Asci from a perithecium obtained by mating a mycelium $S_1$ with mycelium No. 209, which has an origin similar to mycelia Nos. 207–208 (see explanation of pl. 3, C and D). All asci 4 spored except one, *a*, which has 2 large spores. Another large loose spore, shown at *b*, may have belonged in a 3-spored ascus. The perithecia in this culture can not be distinguished morphologically from perithecia of the original $p_2$ parent, *Neurospora tetrasperma*. Compare with A. × 315

only partly developed, others olive green or brown or black. (Pl. 1, C and D.) Sections also show that eight spores are commonly delimited. There can be no doubt that occasionally only six or seven are cut out and that the largest ones originally contain more than one nucleus. Very few asci of this hybrid mature the full complement of eight spores. This, no doubt, is due, however, to the abortion of certain spores after their delimitation.

### MYCELIA FROM FIRST-GENERATION HYBRID ASCOSPORES

The particular culture from which the first-generation ($f_1$) ascospores of the cross *sitophila* × *tetrasperma* were obtained for further work was derived by mating the Columbia University (Cu) strain, which is a haplont A strain of *Neurospora sitophila*, with a monosporous unisexual mycelium $S_1$ of *N. tetrasperma*, which is a haplont B (cf. Shear and Dodge (*9*, *Table 6*)). This culture or cross may be referred to simply as $p_1 \times p_2$: The culture appeared to have matured only a single perithecium at the end of three months. It was on this account that it was chosen to furnish the desired ascospores. Some spores were obtained from the little spore print on the side of the test tube opposite the perithecium, and others were gathered by crushing the fruit body. The ascospores were floated out on the surface of agar in a Petri dish, and some hours later they were heated sufficiently to kill the conidia while at the same time stimulating the ascospores to germinate. Sixty-two germinating ascospores which could be removed separately were originally transferred to agar in other Petri dishes, where 54 of them were measured. They were found to vary somewhat in size, the smallest being 18 by 11 $\mu$, the largest 30 by 15 $\mu$. The average, however, for the 54 spores measured was only 23.1 by 12.6 $\mu$, which is practically the average size of the ascospores of *N. sitophila*. Normal ascospores of *N. tetrasperma* are larger where the average length of 60 spores judged to have been formed in 4-spored asci was 31.6 $\mu$. After examination had shown that only a single ascospore had been selected in each case, the young mycelium was transferred to corn-meal agar tubes. None of the cultures except No. 34 has ever produced perithecia when grown alone. This one fertile culture was probably a mixture resulting from a contamination by accidental transfer of conidia which had not been killed during the heating process. The ascospore transferred measured only 22 by 8 $\mu$, which is too small for a bisexual spore. In any event, the nature of this mycelium can be determined at any time by plating out single conidia, and it need not be considered further at this time.

### FIRST-GENERATION HYBRID CONIDIA

Haplont mycelia of *Neurospora sitophila*, from whatever source obtained, agree in one particular, and that is in the production of an abundance of salmon-pink conidia. Individual strains, however, differ greatly in the number of sterile bulbils or sclerotiumlike bodies which are developed in culture. The Bainier and also the Berkhout strains (*9*, *p. 1029*) produce large numbers of these small bodies in corn-meal agar cultures. Some strains also tend to darken the culture medium. On the other hand, strains like B 704 and the Rose and the Cooley (*9*, *p. 1029*) develop no bulbils or bodies other than conidia,

and the corn-meal agar cultures are not darkened. All of the unisexual mycelia of *N. tetrasperma* (*9, p. 1036*) produce the sterile blackish bodies in great numbers. Some of the bodies are so large as to be easily mistaken for young perithecia. Very few conidia are developed in corn-meal agar cultures. Fertile cultures of this species from bisexual spores invariably develop large numbers of perithecia and some pale salmon-pink conidia, but only a very few of the sterile bodies. The corn-meal agar is not darkened. These facts are mentioned here because of their bearing on the selection and identification of hybrid strains.

The original $p_1 \times p_2$ culture in which perithecia developed, giving the first generation hybrid $f_1$ ascospores, of course contains conidia of each parent species. If, however, the factor for conidia production should be segregated out during the reduction divisions in the ascus at the end of the $F_1$ generation, then monosporous mycelia derived from $f_1$ ascospores should show differences in the characteristics of the $f_1$ conidia formed. The growth of conidia in the $f_1$ cultures was therefore watched with interest. Within a few days most of the cultures developed large numbers of conidia of the *sitophila* parent type. Not a single culture showed the very scanty production of conidia characteristic of *tetrasperma*. In cultures Nos. 1, 4, 5, 13, 19, and perhaps some others, the conidia are somewhat paler, and fewer are produced, suggesting a large element of *tetrasperma* inheritance. As the cultures age, still greater differences are noticeable, particularily with regard to the darkening of the culture medium. These differences seem to be rather constant, as shown in the three sets of subcultures made by transfer of conidia from the original cultures. The following mycelia develop an abundance of the salmon-pink type of conidia without any darkening of the agar: Nos. 2, 8, 9, 12, 14, 17, 22, 23, 25, 27, 35, 40, 41, 44, 45, 49, 56, 58, 62. Other cultures which produce the *sitophila* type of conidia, but which darken the medium and tend to produce many dark-colored sterile bodies, are the following: Nos. 3, 6, 7, 10, 11, 14, 15, 18, 31, 32, 33, 35, 36, 43, 53, 54, 55, 59, 61. These differences with regard to the production of conidia, bulbils, and the darkening of the medium just noted, are possibly no greater than would be found in case an equal number of haplont mycelia of *Neurospora sitophila* were gathered from different parts of the world and grown under similar conditions. No great care was taken to secure a standardized corn-meal agar medium or to place the cultures in exactly the same environment, so that the abundance of conidia produced in any cases reported in this paper is merely suggestive.

### SEXUALITY OF FIRST-GENERATION HYBRID MYCELIA

The sexual nature of the 60 first-generation ($f_1$) hybrid mycelia has been determined as compared with the reciprocal haplonts A and B of *Neurospora sitophila* and $S_6$ and $S_1$ of *N. tetrasperma*. This was done by pairing in culture each mycelium with haplonts $S_6$ and $S_1$. The pairing could also have been made with haplonts A and B of the other parent, *N. sitophila*, and this was done in a number of cases, but the reactions are somewhat slower and perhaps less positive when this is done. In most cases the reactions are strong enough to indicate within a week the sexuality of haplonts grown against $S_6$ and $S_1$. Perithecia develop so slowly in certain combinations as to delay

positive determination. It is to be expected that, wholly aside from the question of heterothallism, certain combination cultures would produce more perithecia than others.

The first-generation ($f_1$) hybrid mycelia which have been classed as haplonts A include Nos. 1, 2, 4, 6, 8, 11, 12, 13, (14), 16, 17, 19, 20, 22, 23, 24, 25, 26, 29, 30, 31, 35, 36, 40, 41, 42, 47, 49, 50, 51, 52, 54, 56, 57, 60, 63. Those $f_1$ mycelia which were found to be of the opposite sex, haplonts B, are Nos. 3, 5, 7, 9, 10, 15, 18, 21, 27, 32, 33, 37, 38, 39, 43, 44, 45, 46, 53, 55, 58, 59, 61, 62. The first-generation ($f_1$) hybrid ascospores were originally selected at random from among those which germinated, yet 36 mycelia are haplonts A and 24 are haplonts B. The spore from which No. 14 was derived was not measured, but it was known to be a large spore. Two other cultures, Nos. 100 and 101, isolated later, were also from large spores, 33 $\mu$ and 29 $\mu$ long, respectively, and it was expected that all three spores would prove to be bisexual. They were, however, found to be unisexual and reacted like haplonts A. This would suggest that while only a single nucleus may have been included in the spores in the ascus, some of the spores failed to mature, so that the additional food was available for an increase in size of certain other spores which did mature. On one occasion two spores of *Neurospora tetrasperma*, 33 $\mu$ and 29.5 $\mu$ long, were proved to be unisexual, and each mycelium corresponds sexually to haplonts A ($S_6$) of this species. Ordinarily spores of *N. tetrasperma* of such a size would be bisexual. The three conditions which may account for great irregularity in the size of spores in an ascus have been discussed recently by the writer (*6*). No doubt bisexual first-generation ($f_1$) hybrid ascospores are occasionally developed, but from the results noted above it is clear that the hybrid structures resulting from crossing of *N. sitophila* and *N. tetrasperma* tend to resemble the *sitophila* parent in the production of unisexual spores as well as in the type of conidia produced.

## RECOVERY OF PARENT TYPES

Haplonts Nos. 1 to 10 of the first-generation hybrid mycelia were grown together in pairs in all possible combinations, to determine whether any combination would result in the development of a pure *sitophila* or *tetrasperma* plant. Here also certain combination cultures produce an abundance of salmon-pink conidia while other combinations develop only a few of the paler type of conidia. Mycelia Nos. 1 and 5, when mated, superficially resemble cultures of *tetrasperma*, and mycelia Nos. 8 and 9 mated in culture resemble the *sitophila* parent. In the type of asci and ascospores produced, a corresponding correlated resemblance was apparent. Details will be given later, but it may be stated here that a great many asci in the perithecia, formed in the cultures where mycelia Nos. 1 and 5 were mated with their reciprocal haplonts of *tetrasperma*, develop large spores with from 2 to 5 spores in each ascus. Very rarely are 8 spores of equal size formed. On the other hand, many asci in the culture in which mycelia Nos. 8 and 9 are mated tend to develop quite regularly 8 spores. Asci which originally delimited fewer than 8 spores have also been found. The back-cross cultures in which either mycelium No. 1 or No. 5 was mated with $p_2$ were particularly selected as a basis for the recovery of the *tetrasperma* parent, and cultures in

which Nos. 8 and 9 were mated together were chosen to furnish spores for the purpose of recovering the *sitophila* parent. Further study may show that the other haplonts might have been selected with more satisfactory results.

### RECOVERY OF SITOPHILA TYPE BY REPEATED SELFING

As previously noted, both mycelia Nos. 8 and 9 derived from first-generation hybrid ascospores show an abundance of conidia of the *sitophila* type. In culture 8 the old conidia are somewhat paler and the agar medium finally becomes slightly darkened. These haplonts are of opposite sex and produce perithecia abundantly when grown together. The asci are long and slender, and 8 spores are usually delimited. (Pl. 2, A and C.) Very rarely, however, one finds an ascus with only 6 or 7 spores. In such an event the result is reflected in the difference in size of the spores in the ascus. The spores containing more than one nucleus at their origin will be considerably larger. At the center in Plate 2, B, is shown an ascus with 5 spores. Two large spores and 1 small one are mature. Another large spore can be seen in the upper end of the ascus. Just below this is a small spore with a large oil globule; both are still hyaline. Each of the 3 large spores probably contains 2 nuclei at its origin, and the 2 small spores only 1 each. Since haplonts 8 and 9 together produce perithecia in which most of the asci are 8 spored, the suggestion is strong that such a segregation may have taken place that haplonts of the pure *sitophila* parent could be selected out at once. Against such an assumption, however, was the evidence presented when the $f_1$ mycelia were backcrossed with mycelia $S_1$ and $S_6$ of the *tetrasperma* ($p_2$) parent. In no case were perithecia formed in the cultures in a way corresponding to the results obtained when the two parents, $p_1$ and $p_2$, were originally crossed.

A sowing of the second-generation ($f_2$) hybrid ascospores (pl. 2, C) resulting from the combination 8+9 ($f_1A \times f_1B$) was made. Of the spores which germinated in such a position as to be easily isolated, 58 were measured and their mycelia grown in the usual way. There appears to be but little variation in the size of the spores chosen, their average size being 20.2 $\mu$ by 13.4 $\mu$. Comparing the spores of the $p_1$ *sitophila* parent (pl. 1, A) with these second-generation ($f_2$) hybrid spores (pl. 2, A to C), it will be seen that if anything the $f_2$ spores tend to be a little shorter or plumper. It should be noted, however, that the measurements given are of mature germinated spores, while the figures show mostly rather immature spores. Superficially, the second-generation ($f_2$) hybrid mycelia Nos. 220 to 277 look much alike except that certain cultures, Nos. 223, 243, 264, 269, 273, 274, and 276, seem to produce fewer conidia which are paler in mass. Mycelia Nos. 224, 229, 234, 245, and 265 are intermediate with respect to the abundance and height of color of the conidia under the conditions in which they have been grown. The other cultures produce an abundance of conidia. The 58 mycelia all proved to be unisexual, and by growing them separately with the reciprocal haplonts $S_6$ and $S_1$ of the *tetrasperma* ($p_2$) parent it has been found that 41 are sexually like haplonts A and 17 are like haplonts B previously referred to.

Growing the mycelia which produce paler and fewer conidia together in pairs shows that some trace of the *Neurospora tetrasperma*

parentage is still to be found in some of the $f_3$ spores. One occasionally finds asci with six or seven spores, certain ones of which will be larger. (Pl. 2, D.) Under the same cultural conditions, the pure *sitophila* parent would show a remarkable uniformity of size and state of maturity of spores in an ascus. (Pl. 1, A.)

Those mycelia of the second ($f_2$) generation which are of opposite sex and which produce conidia most like *Neurospora sitophila* have also been grown together in pairs, with the result that certain combinations produce perithecia with asci and ascospores (pl. 2, E) that would not ordinarily be distinguished morphologically from *N. sitophila*. The asci produced in the culture combination 256+233 are all eight spored.

### RECOVERY OF THE TETRASPERMA TYPE BY REPEATED BACK CROSSING

The first-generation hybrid mycelia Nos. 1 and 5 which produce fewer and paler conidia, as noted previously, and which are of opposite sex were mated in a back-cross combination with haplonts $S_1$ and $S_6$ of the *tetrasperma* ($p_2$) parent. Perithecia develop abundantly within about 10 days. When mycelium No. 1 is back crossed the asci formed tend to produce one or two very large spores (pl. 3, B) with one or more small spores in the same ascus. One of the perithecia examined showed a majority of the asci with one or two giant spores. The largest spore shown in this figure was 57 $\mu$ long. The largest spore found in these back-cross generations measured 62 by 25 $\mu$. Such a large spore was germinated, and its mycelium produced perithecia a majority of whose asci had more than four spores. Cultures from giant spores seem to show no special tendency toward the development of asci with large spores.

Asci in the perithecia resulting from the back-cross combination where mycelium No. 5 was used likewise show a variability in the number, size, time of ripening and markings of the spores. (Pl. 3, A.) Individual perithecia also vary in the types of asci matured. In one case, which is in some contrast with the one shown in this figure, the numbers of spores delimited in the asci present at the time were as follows: 5, 7, 6, 6, 8, 4, 6, 5, 6, 5, 7, 5, 4, 5, 7, 8, 6, 7. The spores in one eight-spored ascus varied in size as follows: 27.5 by 14.5 $\mu$, 26 by 14.5 $\mu$, 22 by 13 $\mu$, 20 by 13 $\mu$, 25.5 by 14.4 $\mu$, 25.5 by 14.4 $\mu$, 20 by 13 $\mu$, and 24 by 13 $\mu$.

Monosporous back-cross mycelia Nos. 200 to 210, inclusive, were derived from selections of spores maturing in the culture in which the $f_1$ mycelium No. 5 was mated or back crossed with mycelium $S_6$ of the *tetrasperma* ($p_2$) parent. It so happened that of all of the spores of various sizes sowed at this time the only ones which germinated and which were so situated as to be readily isolated were comparatively small, ranging in size from about 20 to 27 $\mu$ long. These spores were not actually measured. Contrary to what was expected, these mycelia produce scarcely any conidia, and no perithecia were obtained in the 121 cultures representing all of their possible combinations in pairs, duplicating each combination. When, however, the 11 mycelia were paired in back-cross combinations, it was clear from the results that none of them had lost its sexuality. No perithecia develop in cultures where they are mated with mycelium $S_6$, yet when they are mated with mycelium $S_1$, perithecia are produced abundantly, show-

ing that all of these 11 second-generation back-cross mycelia are of one sex and correspond to haplonts A.

Some months later another sowing was made of the first back-cross ascospores produced in the same culture, and 37 additional mycelia were isolated. Twenty-six of them gave conidia similar to conidia of *Neurospora tetrasperma*. It is a curious fact that 18 of these mycelia were also unisexual and of the same sex as the 11 mycelia previously referred to. The other 8 mycelia were bisexual. Eleven mycelia from spores of this sowing originally produced an abundance of the salmon-pink type of conidia resembling those of *N. sitophila*. Of this number, 10 were unisexual and 1 was bisexual. Of the 10 unisexual mycelia which were judged to produce conidia which were more like *N. sitophila*, 8 mycelia were the same sexually as haplonts B. In every case the classification of a mycelium as to the type of conidia it produced in culture was done long before its sexual nature was known. The data gathered concerning the mycelia from this back-cross combination are given in Table 1. The first 11 mycelia, Nos. 200–210, the ascospores which were not measured, are not included, as they were all of the same sex, haplonts A, and produced scarcely any conidia. As noted in connection with the table, certain mycelia which originally produced conidia in some abundance, when transferred to fresh tubes, developed conidia more like *tetrasperma*. Whether or not further culture work proves the inconsistency of any conclusion which one might be led to draw from an interpretation of the table, it is interesting that the scantiness or abundance with which these back-cross mycelia develop conidia should be even remotely correlated with any sex character.

TABLE 1.—*First back-cross* ($f_1 \times p_2$) *ascospores and their mycelia*

| No. | Size of Spore (μ) | Type of conidia | Sexuality | No. | Size of spore (μ) | Type of conidia | Sexuality |
|---|---|---|---|---|---|---|---|
| 300 | 26.2 by 10.8 | Scanty | Unisexual, A. | 319 | 23.4 by 10.8 | Scanty+ | Unisexual, A. |
| 301 | 23.4 by 10.8 | do | Do. | 320 | 21.6 by 10.8 | Abundant−* | Unisexual, B. |
| 302 | 19.8 by 10.8 | Abundant | Unisexual, B. | 321 | 28.8 by 14.4 | Scanty+ | Bisexual. |
| 303 | 28.8 by 13 | do | Bisexual. | 322 | 21.6 by 10.8 | do | Unisexual, A. |
| 304 | 23.4 by 9 | do | Unisexual, B. | 323 | 21.6 by 10 | do | Do. |
| 305 | 19.8 by 10.8 | Abundant* | Unisexual, A. | 324 | 21.6 by 10.8 | Scanty | Do. |
| 306 | 21 by 10.8 | Scanty | Do. | 325 | 18 by 10.8 | do | Do. |
| 307 | 30 by 15.5 | do | Bisexual. | 326 | 23 by 14.4 | Abundant | Unisexual, B. |
| 308 | 19.8 by 12.3 | Abundant−* | Unisexual, B. | 327 | 21.6 by 10.8 | Abundant−* | Do. |
| 309 | 19.8 by 11.5 | Scanty+ | Unisexual, A. | 328 | 23.4 by 11 | Abundant | Do. |
| 310 | 21.6 by 10.8 | Abundant | Unisexual, B. | 329 | 28.8 by 18 | Scanty+ | Bisexual. |
| 311 | 28.8 by 15 | Scanty+ | Bisexual. | 330 | 28.8 by 14.4 | do | Unisexual, A. |
| 312 | 21.6 by 10.8 | Scanty | Unisexual, A. | 331 | 28.8 by 14.4 | do | Bisexual. |
| 313 | 27.2 by 14.4 | Scanty+ | Bisexual. | 332 | 28.8 by 14.4 | Scanty | Do. |
| 314 | 21 by 10 | do | Unisexual, A. | 333 | 21.6 by 10.8 | Scanty+ | Unisexual, A. |
| 315 | 23.4 by 10.8 | Abundant−* | Do. | 334 | 20.5 by 10.8 | Scanty | Do. |
| 316 | 24.5 by 10.8 | Scanty | Do. | 335 | 21.6 by 9 | Scanty+ | Do. |
| 317 | 21.6 by 10 | Scanty+ | Do. | 336 | 34.2 by 14.4 | do | Bisexual. |
| 318 | 20 by 10.8 | do | Do. | | | | |

* The later subcultures showed only a scanty development of conidia.

The table shows that, with one exception, all of the mycelia which are unisexual were derived from spores under 27 μ long. Spores which approach in size ascospores of the *tetrasperma* parent are commonly bisexual. Further work will be necessary to learn just why most of the small spores whose mycelia develop few conidia are all of the same sex.

Each of the 28 mycelia (Nos. 200 to 210 in addition to the unisexual haplont A mycelia, listed in Table 1) derived from ascospores of the first back-cross mating (pl. 3, A) was mated with $S_1$ of the ancestral parent *tetrasperma*. Most of the matings resulted in the development of asci which as a rule have more than 4 spores. The asci from the mating $207 \times p_2$ (pl. 3, C) contain mostly 5 or 6 spores. The asci in some perithecia from the mating $208 \times p_2$ are quite regularly 4 spored (pl. 3, E); in others 5-spored asci with one or two aborted spores are not uncommon (pl. 3, D). Mating $209 \times p_2$ seems to show that mycelium No. 209 is so nearly like that of $p_2$ in its inheritance that the asci produced by the mating (pl. 4, B) are just as regularly 4 spored as are those of *Neurospora tetrasperma*. In fact, one can find, if persistent, perithecia of *N. tetrasperma* in which two or three 5-spored asci are maturing. (Pl. 4, A.) Of the two perithecia shown in this illustration (pl. 4, A and B), the asci resulting from the second back cross are more regularly 4 spored than are the asci of the ancestor, $p_2$.

The importance of selecting small spores when breeding for purity is well illustrated by the results obtained when one tries to recover the *tetrasperma* type from the second-generation back-cross spores. Asci developed in the back-cross culture $208 \times p_2$ frequently contain, as noted, only four spores. Twenty-six monosporous mycelia, Nos. 400 to 425, were obtained by germinating spores which for the most part came from four-spored asci. When the perithecia developed there was usually the greatest variability in the number of spores in an ascus. In only two cultures did the majority of the asci have four spores. In most cases asci with five to seven spores predominated. Culture 415 was unisexual.

The recovery of the eight-spored *sitophila* type of perithecia by mating in order mycelia of opposite sex, first from the $f_1$, then from the $f_2$ hybrid ascospores, together with the recovery of the four-spored *tetrasperma* type by back crossing, may be looked upon as evidence that these fungi behave in crossing according to Mendelian principles. The remarkable increase in fertility, however, when the recovered *sitophila* type is again crossed with the original *tetrasperma* parent must be taken to indicate that the recovered type is not exactly like the ancestral form of *Neurospora sitophila* unless as a result of the crossing the change is merely one of increased fertility. The same is true with regard to crosses between the original *sitophila* type and the recovered *tetrasperma* type. No doubt, by continued selection along the lines indicated, strains may be recovered which are physiologically as well as morphologically like the original parents.

The importance of the discovery of species of fungi which can be hybridized so readily is increased by the fact that these species at the same time possess such striking diagnostic characters that they are beautifully adapted for the study of the principles of heredity as applied to the fungi. It has taken several decades and great effort on the part of a number of workers to convince the conservatives of the botanic world that certain species of fungi still retain the power to reproduce themselves sexually. No one can observe the most astonishing variability in types of asci developed in the back crosses referred to previously, contrasting this with the great uniformity in ascus

types of the parent species, and still doubt that nuclei from each of the parents had come together and fused in each $F_1$ ascus. Since there can be no doubt that species of the genus Neurospora can be hybridized in the laboratory there is no reason why crossing should not occur in nature. What this may mean as bearing on the great problems of taxonomy and pathology is evident without further discussion at this time.

## SUMMARY

Fertile hybrid perithecia have been produced in culture by crossing two species of the red bread-mold fungi *Neurospora sitophila* and *N. tetrasperma*. The asci of the heterothallic form, *N. sitophila*, are regularly eight spored; and the asci of the homothallic species, *N. tetrasperma*, are commonly four spored, although occasionally asci which mature five or six spores are formed. In this case the smaller spores will be unisexual.

The hybrid perithecia resemble in many respects the fruit bodies of *Neurospora sitophila*. Eight spores are commonly delimited in the asci of the hybrid ascocarps, although asci in which only six or seven spores were delimited have been found. There is great irregularity in the size, markings, time of ripening, and number of spores matured in the ascus. As a rule not all of the spores in an ascus are matured.

The first-generation hybrid mycelia usually develop an abundance of conidia of the *sitophila* type. When eight spores are delimited in an ascus they are all unisexual. In the number of ascospores delimited, size of the spores, and type of conidia produced, the first-generation ($f_1$) hybrids are predominantly like the *sitophila* parent.

Second-generation hybrid perithecia have been produced by mating certain mycelia derived from the first-generation hybrid ascospores. The asci in this second-generation cross are very commonly eight spored, each spore being unisexual. Rarely one finds asci with seven spores, one of which is larger and presumably bisexual. Second-generation hybrid mycelia also show some differences in the type of conidia produced.

By mating selected $f_2$ mycelia of opposite sex, third-generation hybrid ascocarps have been developed. These structures would not be distinguished morphologically from ascocarps of the ancestral parent, $p_1$, *Neurospora sitophila*. Behavior of mycelia from their ascospores show, however, that the *sitophila* type recovered is not exactly like the ancestral parent.

The *tetrasperma* type of perithecium has been recovered by back crossing certain first-generation ($f_1$) hybrid mycelia with the $p_2$ parent form, *N. tetrasperma*, and then again back crossing mycelia derived from ascospores produced in the first back cross with the $p_2$ parent type. Perithecia resulting from certain of these matings regularly show asci with four spores, and these structures resemble morphologically ascocarps of *Neurospora tetrasperma*.

Although in their morphology the recovered types seem to resemble their ancestral parents, the purity of the segregations developed so far is questioned.

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# FURTHER STUDIES ON THE PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD [1]

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## INTRODUCTION

The fruitfulness of the biometric method of attack on the problem of field heterogeneity—one of the greatest sources of difficulty in the interpretation of the results of all experiments involving plot tests—has been shown by a series of papers cited in publications readily accessible to agriculturists (*2*, *3*, *4*, *5*, *6*).[2]

In an earlier investigation (*7*) the writers considered the permanence of the differences in the plots of one experimental tract, comprising 46 plots, at the Huntley (Mont.) Field Station.

By applying the method of interannual correlation (*3*) the writers demonstrated that during the period from 1911 to 1919, inclusive, plots which showed a higher yield in one year were generally characterized by a measurably higher yield in subsequent years, whereas those which fell below the average yield in a given year generally proved measurably inferior in other years.

The crops grown during the nine years covered by the first investigation comprised sugar beets, alfalfa, ear corn, oats, silage corn, and barley. Altogether, 19 crop records (including the various cuttings of alfalfa and the separate and combined yields of grain and straw, in the cereals) were available. Since the report of these investigations appeared, 6 crops, giving 10 measures of yield, have been grown on this land. These were silage corn, barley, alfalfa (three years), and silage corn.

The purposes of the present investigation are: (1) To consider the relationship between the yield of these various crops and the other crops grown on these plots during the whole period that the uniform-cropping experiments were under way—that is, from 1911 to 1925, inclusive; (2) to suggest physical explanations for certain of the biological results; and (3) to indicate the bearing of these results on the problem of the technic of agricultural experimentation.

## MATERIALS

While the experimental tract was briefly described in an earlier paper (*7*), certain of the features must be noted in somewhat greater detail to make possible a full understanding of the results here presented.

[1] Received for publication Sept. 17, 1927; issued February, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 40.

This consideration of materials may fall logically into two parts: (1) A description of features of the experimental area, and (2) a discussion of the agronomic details of the crop yields.

The Huntley Field Station is located in the Yellowstone Valley on land having a very slight and uniform slope to the north.

The plots involved in this experiment occupy a rectangular block of land known as Series II and III of field B. Field B is triangular and lies between the main canal of the Huntley Reclamation Project and an open drain ditch known as Custer Coulee. Series II and III occupy the center of the north side of this triangle.

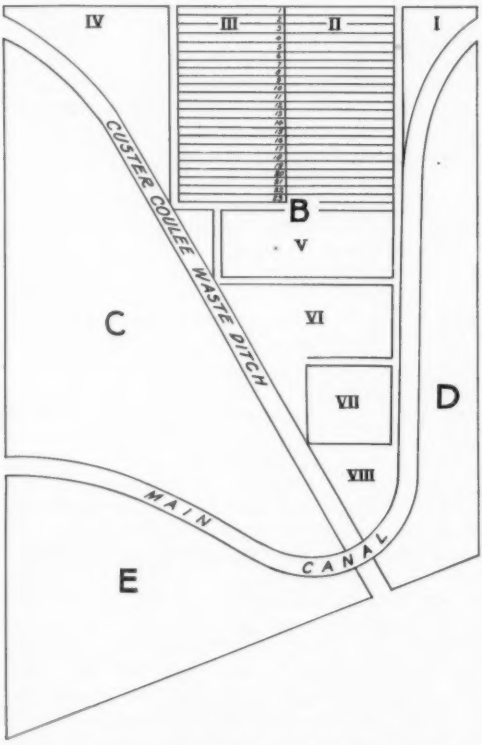


FIG. 1.—Diagram of fields B, C, D, and E of the Huntley (Mont.) Field Station, showing the locations of the plots of Series II and III of field B as related to the irrigation and drainage canals

The map (fig. 1) shows the form and relative position of the 46 plots which constitute Series II and III. Each plot measures 23.3 by 317 feet and contains approximately 0.17 acre. The two series are separated by a narrow alleyway 5 feet wide; the entire block of ground is 639 feet (east and west) by 536 feet (north and south). The slope to the north is 1 foot in 536 feet, while the slope to the west is 2.5 feet in 639 feet. The northeast corner is therefore 3.5 feet lower than the southeast corner. This slope is adequate for the distribution of irrigation water by flooding, as for alfalfa and grain, or by furrows, as for sugar beets and corn. Irrigation water is distributed from a shallow ditch running along the east side of Series II from south to north. When the ground is occupied by a cultivated crop, such as corn, the irrigation water runs between the rows across both series. When the land is planted with grain or alfalfa, another shallow ditch is made in the alleyway between the two series, and each series is flooded from the ditch along its east side.

Some slope of the land is necessary for the distribution of water, but it is found to be difficult to establish and maintain a uniform surface slope over the entire field. An effort is made to smooth the surface each time the land is prepared for a new crop, but slight inequalities of slope may persist. Furthermore, some soil movement may take place through wind action between the time the field is leveled for planting and the time the crop is grown to a size that affords protection for the soil. Even when the land is well stabilized by a crop such as alfalfa, wind may carry soil from adjacent fields or roads, thus making the surface to some extent uneven. Any such irregularities may have a direct influence on the distribution of irrigation water and consequently on the depth of penetration even where the physical conditions of the soil of the root zone are substantially uniform. These irregularities in the soil surface must have some influence upon the character of the crop growth. Growth conditions may be affected adversely either where the surface is slightly high, so that too little water enters the root zone, or where the surface is slightly low, so that water collects and keeps the surface soil saturated too long. The indications are that, with the quantities of irrigation water that have been used on this field, more injury has resulted from local deficiencies of water than from local surpluses. These differences of surface level do not occur in the same spots from year to year, because they are the result of accidental conditions. Consequently, differences of yield from year to year may be due in part to these slight surface irregularities.

The question of the relation of the field to water supply and drainage—factors which are believed to be of importance in determining some of the results reported here—will now be considered.

The southeast corner of Series II, the east series, is about 80 feet from the main canal, and the southwest corner of Series III is about 50 feet from Custer Coulee. The main project canal carries normally during the irrigation season about 400 second-feet of water. The water surface in the canal is about 4 feet above the high corner of the field. It is evident from surface conditions, as well as from borings made between the canal and the field, that there is extensive seepage from the canal into the subsoil of the field. The volume of this seepage has been larger in recent years than it was in the earlier years of the cropping experiments, probably because the canal bank has been worn away by internal erosion, exposing a stratum of sandy subsoil that underlies the canal and part of the field.

As early as 1921 it was observed that in some parts of the field the subsoil was saturated late in the season not far below the surface. In the spring of 1922 an observation well was established near the center of the south line of the field, at the end of the alleyway that separates the two series. In April, when the well was put down, the saturated zone was 7 feet below the surface. During the irrigation season the underground water rose until in October it stood at 2.1 feet below the ground surface. Each year since 1922 a similar condition has been observed. The underground water rises during the irrigation season and falls again during the following winter. Other observation wells were sunk in 1923 along the east and north sides of the field. From these it has been learned that the zone of

subsoil saturation extends under the whole field. The slope of this saturated zone is rather greater than that of the surface soil, so that while it rises nearly to the soil surface along the east side of the field (Series II) it remains 4 to 5 feet below the surface along the west side. It seems obvious that this zone of saturated subsoil as it approaches the surface must restrict the roots of the plants and to some extent influence crop growth. At some stages this influence may be beneficial, since the plants may be able to utilize this subsoil water. At higher stages or with such a deep-rooted crop as alfalfa the influence may be injurious.

It is believed that in the earlier years of these uniform-cropping experiments the subsoil water did not rise so close to the surface as it has recently, although no detailed observations were made prior to 1922. It is also believed that the effects of the subsoil saturation have been more pronounced on the plots of Series II than on those of Series III. It seems probable that the negative correlations reported in this paper between the alfalfa yields of 1912–1914 and those of 1922–1924 may be associated with the unequal effect on the two series of plots of the rising zone of subsoil saturation.

The following notes with respect to the field conditions and crops grown each year may contribute to a better understanding of the yields reported and analyzed in this paper.

The detailed history of the field prior to 1910 is not definitely known. Probably it was first broken from the original prairie sod in the spring of 1908. In 1909 it was planted to sugar beets, but the crop was destroyed by hail in the late summer. In 1910 the field came under experimental control. At that time the major portion was sown to oats, which produced a yield of 66 bushels per acre. That year a small tract in the southeast corner of the field was used as an implement park and stack yard and was not put into crops. This area occupied about two-thirds of the length of the first five plots of Series II. Possibly this difference in treatment in 1910 may have influenced the crop yields of 1911, but it seems probable that such influence was not great.

Year by year the crops were as follows:

1911. Sugar beets. (See above.)

1912. Montana common alfalfa was seeded on May 18 without a nurse crop. The first crop was cut July 18, but the plots were not harvested separately. The total yield from the two series was 6,840 pounds. The second cutting (reported in Table 2 as "1912, alfalfa total") was made September 10.

1913. Alfalfa I was cut June 20; Alfalfa II was cut August 2; the third cutting was made September 21. While this crop was curing in the field a severe windstorm occurred, and the hay was so badly mixed that the plot yields could not be determined. It was not possible even to obtain the total yield, as part of the hay was blown completely off the field.

1914. Alfalfa I was cut June 15; Alfalfa II was cut July 7; Alfalfa III was cut September 20. The land was subsequently plowed.

1915. Ear corn, variety Northwestern Dent, was planted May 20. Harvest was begun September 20, but was delayed for several weeks by stormy weather. The stover weights were not taken, because of storm injury during harvest.

1916. Ear corn, variety Northwestern Dent, was planted May 26. The soil was very dry at planting time and the crop was slow in starting. Harvesting was delayed by wet weather until December 1, and again the injury to the stover was so great that it seemed inadvisable to weigh it by plots.

1917. Oats, variety Banner, were sown May 28 and harvested August 17. No exceptional conditions were noted. The crop yield was very good.

1918. Silage corn, variety Northwestern Dent, was planted May 20 and cut September 18. No exceptional conditions were noted.

1919. Barley, variety Trebi, was sown May 10 and harvested September 12. Because of drought, an irrigation was necessary to germinate the seed. Only a fair stand was obtained.

1920. Silage corn, variety Northwestern Dent. On July 4 water from Custer Coulee caused an overflow of the main canal and flooded the experimental field. This set back the growth of the corn crop and probably reduced the yield, but so far as could be observed the injury was fairly uniform on the two series. The crop was harvested September 16.

1921. Barley, variety Trebi. A good yield was obtained and approximately a normal grain-straw ratio for this variety.

1922. Alfalfa, variety Grimm, was seeded with the barley in May, 1921, but owing to severe grasshopper injury it was necessary to reseed in August, 1921. In 1922 the first and second cuttings of alfalfa were not harvested by separate plots because of the prevalence and irregular distribution of weeds and grasshopper injury. The alfalfa reported for 1922 in Table 2 is the third crop, cut September 27. The yields for the first cutting, June 23, were: Series II, 8,090 pounds, or 1.03 tons per acre; Series III, 7,000 pounds, or 0.90 ton per acre. The yields for the second cutting, August 4, were: Series II, 8,050 pounds, or 1.03 tons per acre; Series III, 7,950 pounds, or 1.02 tons per acre.

1923. Alfalfa. The first cutting was made June 15. The second cutting, made August 3, was so badly lodged by wind and rain that it was not practicable to weigh the plot yields separately. The yield of Series II was 15,730 pounds, or 2.01 tons per acre; the yield of Series III was 16,911 pounds, or 2.16 tons per acre. The third cutting was not made until November 20, about six weeks after it was ready to cut. This delay was due to continuously unfavorable weather, and although there was appreciable loss of weight because of the delay it was not apparent that any lack of uniformity in yield resulted.

1924. Alfalfa. The first crop was cut June 25. It was so badly lodged by wind and rain that the plots could not be weighed separately. Series II yielded 18,660 pounds, or 2.39 tons per acre, and Series III yielded 19,500 pounds, or 2.50 tons per acre. The second cutting was made August 5. The third cutting was made September 26. The hay from plots B–II, 1 to 14, inclusive, was weighed on October 2. The hay from the remaining plots of B–II and all the other plots was weighed October 16. The delay was due to a heavy rain, which may have caused some loss of leaves occasioned by the necessary turning and drying.

1925. Silage corn, variety Northwestern Dent, harvested September 12. Apparently a normal crop and good yield.

The yields of the various crops grown during the period 1911–1919 are set forth in Table 3 of a previous paper (7). Those for the period 1920–1925 are shown in Table 1 of the present paper. This table gives the yields in terms of pounds per plot. For the convenience of agriculturists the average yields in pounds per plot and the equivalent yields per acre in conventional units are shown in Table 2.

TABLE 1.—*Crop yields in the uniform-cropping experiments at Huntley, Mont., 1920–1925*

| Series and plot No. | Yield per plot (pounds) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Silage corn, 1920 | Barley, 1921 | | | Alfalfa | | | | | Silage corn, 1925 |
| | | Grain | Straw | Total | III, 1922 | I, 1923 | III, 1923 | II, 1924 | III, 1924 | |
| Series II: | | | | | | | | | | |
| 1 | 2,230 | 506 | 344 | 850 | 420 | 530 | 270 | 450 | 250 | 4,940 |
| 2 | 2,405 | 571 | 389 | 960 | 520 | 450 | 220 | 460 | 290 | 3,820 |
| 3 | 2,800 | 554 | 376 | 930 | 390 | 640 | 280 | 550 | 280 | 3,570 |
| 4 | 2,720 | 559 | 361 | 920 | 340 | 510 | 260 | 500 | 330 | 3,600 |
| 5 | 2,470 | 569 | 381 | 950 | 390 | 620 | 290 | 650 | 290 | 4,150 |
| 6 | 2,290 | 512 | 388 | 900 | 390 | 540 | 250 | 540 | 340 | 4,490 |
| 7 | 2,320 | 552 | 348 | 900 | 400 | 600 | 310 | 680 | 280 | 4,280 |
| 8 | 2,335 | 514 | 326 | 840 | 390 | 510 | 250 | 530 | 310 | 4,120 |
| 9 | 2,380 | 499 | 341 | 840 | 410 | 540 | 290 | 670 | 330 | 4,400 |
| 10 | 2,655 | 548 | 372 | 920 | 490 | 545 | 280 | 590 | 370 | 4,110 |
| 11 | 2,410 | 559 | 341 | 900 | 480 | 730 | 360 | 700 | 310 | 4,760 |
| 12 | 2,560 | 598 | 312 | 910 | 500 | 610 | 280 | 610 | 400 | 4,510 |
| 13 | 2,490 | 547 | 303 | 850 | 450 | 670 | 330 | 740 | 400 | 4,600 |
| 14 | 2,560 | 550 | 280 | 830 | 440 | 570 | 290 | 630 | 450 | 4,140 |
| 15 | 2,300 | 509 | 261 | 770 | 400 | 660 | 410 | 800 | 380 | 4,150 |
| 16 | 2,590 | 566 | 334 | 900 | 430 | 560 | 300 | 640 | 430 | 4,720 |
| 17 | 2,490 | 634 | 416 | 1,050 | 420 | 630 | 310 | 700 | 350 | 4,550 |
| 18 | 2,550 | 616 | 374 | 990 | 430 | 550 | 250 | 550 | 370 | 4,340 |
| 19 | 2,610 | 568 | 342 | 910 | 340 | 640 | 310 | 600 | 280 | 4,390 |
| 20 | 2,430 | 571 | 379 | 950 | 360 | 630 | 260 | 560 | 230 | 4,420 |
| 21 | 2,180 | 546 | 344 | 890 | 320 | 640 | 290 | 650 | 290 | 4,520 |
| 22 | 2,360 | 521 | 239 | 760 | 230 | 550 | 210 | 530 | 310 | 4,320 |
| 23 | 1,790 | 452 | 288 | 740 | 200 | 540 | 240 | 530 | 260 | 4,180 |
| Series III: | | | | | | | | | | |
| 1 | 2,240 | 466 | 304 | 770 | 260 | 645 | 350 | 720 | 360 | 4,690 |
| 2 | 2,110 | 485 | 325 | 810 | 330 | 540 | 360 | 660 | 410 | 3,590 |
| 3 | 2,535 | 519 | 351 | 870 | 340 | 750 | 370 | 720 | 380 | 3,070 |
| 4 | 2,480 | 493 | 307 | 800 | 350 | 490 | 320 | 590 | 410 | 3,140 |
| 5 | 2,300 | 498 | 342 | 840 | 395 | 670 | 340 | 790 | 400 | 3,940 |
| 6 | 2,215 | 436 | 334 | 770 | 350 | 590 | 340 | 640 | 350 | 3,960 |
| 7 | 2,150 | 502 | 338 | 840 | 420 | 720 | 330 | 800 | 400 | 4,170 |
| 8 | 2,220 | 497 | 303 | 800 | 390 | 500 | 360 | 660 | 420 | 3,880 |
| 9 | 2,400 | 476 | 304 | 780 | 395 | 760 | 320 | 820 | 390 | 3,970 |
| 10 | 2,460 | 489 | 311 | 800 | 305 | 590 | 290 | 620 | 420 | 3,270 |
| 11 | 2,245 | 465 | 305 | 770 | 290 | 620 | 360 | 780 | 380 | 4,105 |
| 12 | 2,330 | 492 | 298 | 790 | 410 | 550 | 340 | 550 | 390 | 4,495 |
| 13 | 2,400 | 506 | 304 | 810 | 380 | 600 | 280 | 750 | 330 | 4,825 |
| 14 | 2,430 | 526 | 314 | 840 | 370 | 550 | 270 | 590 | 360 | 4,390 |
| 15 | 2,300 | 523 | 297 | 820 | 340 | 630 | 290 | 690 | 340 | 4,380 |
| 16 | 2,530 | 501 | 289 | 790 | 305 | 500 | 280 | 530 | 380 | 4,580 |
| 17 | 2,540 | 515 | 305 | 820 | 380 | 640 | 300 | 670 | 360 | 4,520 |
| 18 | 2,480 | 537 | 273 | 810 | 365 | 600 | 240 | 570 | 390 | 4,290 |
| 19 | 2,610 | 505 | 285 | 790 | 375 | 740 | 290 | 720 | 380 | 4,620 |
| 20 | 2,610 | 531 | 309 | 840 | 370 | 650 | 310 | 600 | 420 | 4,510 |
| 21 | 2,480 | 542 | 288 | 830 | 440 | 640 | 340 | 790 | 400 | 4,770 |
| 22 | 1,940 | 523 | 317 | 840 | 345 | 680 | 270 | 620 | 420 | 4,850 |
| 23 | 2,200 | 530 | 300 | 830 | 410 | 670 | 320 | 810 | 420 | 5,230 |

TABLE 2.—*Average yields of crops grown in the uniform-cropping experiments at Huntley, Mont., 1911–1925*

[Yields per acre (last column) are stated in tons except for ear corn, barley grain, and oats grain, which are in bushels]

| Year | Crop | Per plot (pounds) | Per acre |
|---|---|---|---|
| 1911 | Sugar beets | 4,179.00 | 12.29 |
| 1912 | Alfalfa (total) | 356.54 | 1.04 |
| 1913 | Alfalfa I | 541.41 | 1.59 |
| | Alfalfa II | 483.26 | 1.42 |
| | Alfalfa I and II | 1,024.67 | 3.01 |
| 1914 | Alfalfa I | 489.13 | 1.44 |
| | Alfalfa II | 499.34 | 1.47 |
| | Alfalfa I and II | 988.47 | 2.91 |
| | Alfalfa III | 471.95 | 1.38 |
| | Alfalfa I to III | 1,460.43 | 4.29 |
| 1915 | Ear corn | 522.58 | 42.70 |
| 1916 | do | 396.15 | 32.40 |
| 1917 | Oats, Grain | 555.80 | 102.10 |
| | Oats, Straw | 521.54 | 1.53 |
| | Oats, Total | 1,077.34 | 3.16 |
| 1918 | Silage corn | 3,175.43 | 9.34 |
| 1919 | Barley, Grain | 358.19 | 43.80 |
| | Barley, Straw | 230.50 | .67 |
| | Barley, Total | 588.69 | 1.73 |
| 1920 | Silage corn | 2,394.13 | 7.04 |
| 1921 | Barley, Grain | 525.60 | 64.20 |
| | Barley, Straw | 324.82 | .95 |
| | Barley, Total | 850.43 | 2.50 |
| 1922 | Alfalfa III | 379.67 | 1.12 |
| 1923 | Alfalfa I | 603.91 | 1.77 |
| | Alfalfa III | 300.22 | .88 |
| | Alfalfa I and III | 904.13 | 2.66 |
| 1924 | Alfalfa II | 642.39 | 1.89 |
| | Alfalfa III | 357.39 | 1.05 |
| | Alfalfa II and III | 999.78 | 2.94 |
| 1925 | Silage corn | 4,267.93 | 12.55 |

## PRESENTATION OF RESULTS

The correlations between the crops grown from 1911 to 1919, inclusive, have been set forth in Tables 4 to 6 of an earlier publication (7).

With the new materials at hand it is worth while to review all of the correlations available for the entire period of 15 years over which these uniform-cropping experiments have been continued.

The correlations between each crop yield of the period 1920 to 1925 and the yields of each of the crops grown from 1911 to 1925, both inclusive, are shown in Table 3.

The publication of one of the present tables in condensed form and the appearance of these tables in two different places renders the examination of the results extremely difficult. A graphic method of representation, therefore, has been adopted.

TABLE 3.—*Correlation between the yields of various crops in the uniform-cropping experiments at Huntley, Mont.*

| Year | Crop | Item | Sugar beets, 1911 | Alfalfa | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total, 1912 | I, 1913 | II, 1913 | I and II, 1913 | I, 1914 | II, 1914 | I and II, 1914 |
| 1920 | Silage corn | Correlation | −0.237 ± .093 | +0.074 ± .098 | +0.117 ± .098 | +0.189 ± .095 | +0.179 ± .096 | +0.164 ± .096 | +0.164 ± .096 | +0.177 ± .096 |
| | | r/Er | 2.52 | .75 | 1.20 | 1.97 | 1.86 | 1.70 | 1.69 | 1.84 |
| 1921 | Barley, Grain | Correlation | +.081 ± .098 | +.001 ± .099 | +.473 ± .077 | +.219 ± .094 | +.408 ± .082 | +.529 ± .071 | +.286 ± .091 | +.432 ± .080 |
| | | r/Er | .82 | .01 | 6.13 | 2.31 | 4.93 | 7.39 | 3.13 | 5.35 |
| | Barley, Straw | Correlation | −.350 ± .087 | +.219 ± .094 | +.415 ± .082 | +.189 ± .095 | +.356 ± .086 | +.492 ± .075 | +.408 ± .082 | +.484 ± .076 |
| | | r/Er | 4.02 | 2.31 | 5.04 | 1.97 | 4.10 | 6.54 | 4.93 | 6.36 |
| | Barley, Total | Correlation | −.144 ± .097 | +.121 ± .097 | +.515 ± .073 | +.237 ± .093 | +.443 ± .079 | +.591 ± .064 | +.398 ± .083 | +.528 ± .071 |
| | | r/Er | 1.48 | 1.24 | 7.05 | 2.52 | 5.55 | 9.15 | 4.76 | 7.38 |
| 1922 | Alfalfa III | Correlation | −.197 ± .095 | +.195 ± .095 | +.056 ± .099 | +.203 ± .095 | +.151 ± .097 | +.292 ± .090 | +.115 ± .098 | +.214 ± .094 |
| | | r/Er | 2.06 | 2.04 | .57 | 2.13 | 1.55 | 3.21 | 1.18 | 2.26 |
| 1923 | Alfalfa I | Correlation | +.082 ± .098 | −.011 ± .099 | −.066 ± .099 | −.207 ± .095 | −.159 ± .096 | −.224 ± .094 | −.264 ± .092 | −.265 ± .092 |
| | | r/Er | .83 | .11 | .67 | 2.17 | 1.64 | 2.38 | 2.86 | 2.87 |
| | Alfalfa III | Correlation | −.081 ± .098 | +.150 ± .097 | −.224 ± .094 | −.333 ± .088 | −.326 ± .088 | −.396 ± .083 | −.438 ± .080 | −.452 ± .079 |
| | | r/Er | .82 | 1.54 | 2.37 | 3.77 | 3.67 | 4.73 | 5.45 | 5.72 |
| | Alfalfa I and III | Correlation | +.025 ± .099 | +.055 ± .099 | −.143 ± .097 | −.292 ± .090 | −.254 ± .092 | −.332 ± .088 | −.379 ± .085 | −.386 ± .084 |
| | | r/Er | .26 | .55 | 1.47 | 3.22 | 2.73 | 3.75 | 4.45 | 4.56 |
| 1924 | Alfalfa II | Correlation | +0 ± .099 | +.099 ± .098 | −.272 ± .092 | −.362 ± .08 | −.371 ± .085 | −.394 ± .083 | −.369 ± .085 | −.412 ± .082 |
| | | r/Er | 0 | 1.00 | 2.95 | 4.19 | 4.33 | 4.69 | 4.30 | 4.99 |
| | Alfalfa III | Correlation | −.026 ± .099 | −.047 ± .099 | −.556 ± .068 | −.279 ± .091 | −.492 ± .075 | −.540 ± .070 | −.544 ± .069 | −.586 ± .065 |
| | | r/Er | .26 | .48 | 8.09 | 3.04 | 6.53 | 7.67 | 7.77 | 8.98 |
| | Alfalfa II and III | Correlation | −.010 ± .099 | +.054 ± .099 | −.435 ± .080 | −.388 ± .084 | −.483 ± .076 | −.520 ± .072 | −.503 ± .074 | −.553 ± .069 |
| | | r/Er | .10 | .54 | 5.41 | 4.59 | 6.34 | 7.18 | 6.79 | 8.01 |
| 1925 | Silage corn | Correlation | +.512 ± .073 | −.572 ± .066 | +.131 ± .097 | +.096 ± .098 | +.134 ± .097 | +.074 ± .098 | +.022 ± .099 | +.051 ± .099 |
| | | r/Er | 6.99 | 8.57 | 1.34 | .98 | 1.37 | .75 | .20 | .51 |

| Year | Crop | Item | Alfalfa—Continued | | Ear corn | | Oats | | | Silage corn, 1918 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | III, 1914 | I to III, 1914 | 1915 | 1916 | Grain, 1917 | Straw, 1917 | Total, 1917 | |
| 1920 | Silage corn | Correlation | +0.111 ± .098 | +0.171 ± .096 | −0.478 ± .076 | +0.105 ± .098 | +0.251 ± .093 | +0.983 ± .098 | +0.185 ± .096 | −0.152 ± .097 |
| | | r/Er | 1.13 | 1.77 | 6.24 | 1.07 | 2.69 | .84 | 1.93 | 1.56 |
| 1921 | Barley, Grain | Correlation | +.248 ± .093 | +.411 ± .082 | −.131 ± .097 | +.401 ± .083 | +.268 ± .092 | +.143 ± .097 | +.238 ± .093 | +.011 ± .099 |
| | | r/Er | 2.65 | 4.97 | 1.34 | 4.81 | 2.90 | 1.47 | 2.54 | .11 |
| | Barley, Straw | Correlation | +.343 ± .087 | +.048 ± .099 | −.035 ± .099 | +.400 ± .083 | +.331 ± .088 | +.278 ± .091 | +.370 ± .085 | +.036 ± .099 |
| | | r/Er | 3.91 | .48 | .35 | 4.79 | 3.74 | 3.03 | 4.31 | .36 |
| | Barley, Total | Correlation | +.339 ± .087 | +.573 ± .073 | −.098 ± .098 | +.463 ± .078 | +.345 ± .087 | +.240 ± .093 | +.348 ± .087 | +.027 ± .099 |
| | | r/Er | 3.85 | 7.00 | 1.00 | 5.93 | 3.93 | 2.56 | 3.99 | .27 |
| 1922 | Alfalfa III | Correlation | +.057 ± .099 | +.184 ± .096 | −.058 ± .099 | +.192 ± .095 | +.148 ± .097 | +.397 ± .083 | +.369 ± .085 | −.126 ± .097 |
| | | r/Er | .57 | 1.91 | .59 | 2.01 | 1.53 | 4.74 | 4.30 | 1.29 |
| 1923 | Alfalfa I | Correlation | −.125 ± .097 | −.244 ± .093 | −.127 ± .097 | −.260 ± .092 | −.187 ± .095 | +.095 ± .098 | −.020 ± .099 | +.101 ± .098 |
| | | r/Er | 1.28 | 2.61 | 1.30 | 2.80 | 1.95 | .97 | .20 | 1.03 |
| | Alfalfa III | Correlation | −.142 ± .097 | −.394 ± .083 | −.101 ± .098 | −.439 ± .080 | −.405 ± .083 | +.141 ± .097 | −.092 ± .098 | −.127 ± .097 |
| | | r/Er | 1.45 | 4.70 | 1.02 | 5.47 | 4.87 | 1.45 | .94 | 1.29 |
| | Alfalfa I and III | Correlation | −.152 ± .097 | −.346 ± .087 | −.136 ± .097 | −.376 ± .085 | −.308 ± .089 | +.130 ± .097 | −.054 ± .099 | +.021 ± .099 |
| | | r/Er | 1.56 | 3.95 | 1.39 | 4.40 | 3.43 | 1.33 | .54 | .21 |
| 1924 | Alfalfa II | Correlation | −.214 ± .094 | −.385 ± .084 | −.021 ± .099 | −.459 ± .078 | −.381 ± .085 | −.025 ± .099 | −.206 ± .095 | −.130 ± .097 |
| | | r/Er | 2.26 | 4.54 | .22 | 5.85 | 4.48 | .26 | 2.16 | 1.33 |
| | Alfalfa III | Correlation | −.363 ± .086 | −.565 ± .067 | +0.42 ± .099 | −.561 ± .068 | −.430 ± .081 | +.040 ± .099 | −.181 ± .096 | −.154 ± .097 |
| | | r/Er | 4.21 | 8.35 | .42 | 8.25 | 5.30 | .40 | 1.88 | 1.58 |
| | Alfalfa II and III | Correlation | −.312 ± .089 | −.524 ± .072 | +.001 ± .099 | −.578 ± .066 | −.465 ± .077 | −.002 ± .099 | −.230 ± .094 | −.162 ± .096 |
| | | r/Er | 3.48 | 7.26 | .01 | 8.73 | 5.96 | .02 | 2.44 | 1.67 |
| 1925 | Silage corn | Correlation | −.027 ± .099 | +.031 ± .099 | +.107 ± .098 | +.192 ± .095 | +.176 ± .096 | −.030 ± .099 | +.064 ± .099 | +.093 ± .098 |
| | | r/Er | .28 | .31 | 1.09 | 2.01 | 1.83 | .30 | .65 | .95 |

TABLE 3.—*Correlation between the yields of various crops in the uniform-cropping experiments at Huntley, Mont.*—Continued

| Year | Crop | Item | Barley | | | Silage corn, 1920 | Barley | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Grain, 1919 | Straw, 1919 | Total, 1919 | | Grain, 1921 | Straw, 1921 | Total, 1921 |
| 1920 | Silage corn | Correlation | −0.092 ±.098 | −0.274 ±.091 | −0.195 ±.095 | | +0.532 ±.071 | +0.206 ±.093 | +0.435 ±.080 |
| | | r/Er | .93 | 2.98 | 2.04 | | 7.46 | 2.16 | 5.39 |
| 1921 | Barley (Grain) | Correlation | −.184 ±.096 | −.282 ±.091 | −.253 ±.093 | +.532 ±.071 | | +.495 ±.075 | +.877 ±.022 |
| | | r/Er | 1.92 | 3.08 | 2.72 | 7.46 | | 6.60 | 38.3 |
| | Barley (Straw) | Correlation | +.348 ±.087 | +.203 ±.095 | +.308 ±.090 | +.206 ±.095 | +.495 ±.075 | | +.851 ±.027 |
| | | r/Er | 3.99 | 2.13 | 3.42 | 2.16 | 6.60 | | 31.2 |
| | Barley (Total) | Correlation | +.061 ±.098 | −.057 ±.099 | +.017 ±.099 | +.435 ±.080 | +.877 ±.022 | +.851 ±.027 | |
| | | r/Er | .82 | .58 | .17 | 5.39 | 38.3 | 31.2 | |
| 1922 | Alfalfa III | Correlation | +.282 ±.091 | +.184 ±.096 | +.259 ±.092 | +.376 ±.085 | +.534 ±.071 | +.372 ±.085 | +.528 ±.071 |
| | | r/Er | 3.08 | 1.92 | 2.80 | 4.41 | 7.51 | 4.35 | 7.39 |
| 1923 | Alfalfa I | Correlation | −.021 ±.099 | −.001 ±.099 | −.012 ±.099 | +.018 ±.099 | +.005 ±.099 | −.091 ±.098 | −.047 ±.099 |
| | | r/Er | .21 | .01 | .12 | .18 | .05 | .93 | .47 |
| | Alfalfa III | Correlation | +.232 ±.094 | +.078 ±.098 | +.175 ±.096 | −.109 ±.098 | −.296 ±.090 | −.198 ±.096 | −.265 ±.092 |
| | | r/Er | 2.47 | .79 | 1.82 | 1.11 | 3.26 | 2.05 | 2.87 |
| | Alfalfa I and III | Correlation | +.082 ±.098 | +.032 ±.099 | +.064 ±.099 | −.032 ±.099 | −.120 ±.097 | −.133 ±.097 | −.146 ±.097 |
| | | r/Er | .83 | .33 | .65 | .32 | 1.23 | 1.37 | 1.50 |
| 1924 | Alfalfa II | Correlation | +.130 ±.097 | +.065 ±.099 | +.109 ±.098 | −.144 ±.097 | −.193 ±.095 | −.239 ±.093 | −.249 ±.093 |
| | | r/Er | 1.33 | .65 | 1.11 | 1.47 | 2.02 | 2.55 | 2.67 |
| | Alfalfa III | Correlation | +.024 ±.099 | −.058 ±.099 | −.547 ±.069 | +.073 ±.098 | −.155 ±.097 | −.411 ±.082 | −.321 ±.089 |
| | | r/Er | .24 | .58 | 7.85 | .74 | 1.60 | 4.97 | 3.60 |
| | Alfalfa II and III | Correlation | +.108 ±.098 | +.024 ±.099 | −.146 ±.097 | −.077 ±.098 | −.209 ±.095 | −.351 ±.087 | −.320 ±.089 |
| | | r/Er | 1.10 | .24 | 1.50 | .78 | 2.20 | 4.02 | 3.59 |
| 1925 | Silage corn | Correlation | −.283 ±.091 | −.191 ±.095 | −.535 ±.070 | −.167 ±.096 | +.194 ±.095 | −.152 ±.097 | +.033 ±.099 |
| | | r/Er | 3.10 | 1.99 | 7.54 | 1.73 | 2.02 | 1.57 | .33 |

| Year | Crop | Item | Alfalfa | | | | | | | Silage corn, 1925 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | III, 1922 | I, 1923 | III, 1923 | I and III, 1923 | II, 1924 | III, 1924 | II and III, 1924 | |
| 1920 | Silage corn | Correlation | +0.376 ±.085 | +0.018 ±.099 | −0.109 ±.098 | −0.032 ±.099 | −0.144 ±.097 | +0.073 ±.098 | −0.077 ±.098 | −0.167 ±.096 |
| | | r/Er | 4.41 | .18 | 1.11 | .32 | 1.47 | .74 | .78 | 1.73 |
| 1921 | Barley (Grain) | Correlation | +.534 ±.071 | +.005 ±.099 | −.296 ±.090 | −.120 ±.097 | −.193 ±.095 | −.155 ±.097 | −.209 ±.095 | +.194 ±.095 |
| | | r/Er | 7.51 | .05 | 3.26 | 1.23 | 2.02 | 1.60 | 2.20 | 2.02 |
| | Barley (Straw) | Correlation | +.372 ±.085 | −.091 ±.098 | −.157 ±.096 | −.133 ±.097 | −.239 ±.093 | −.411 ±.082 | −.351 ±.087 | −.152 ±.097 |
| | | r/Er | 4.35 | .93 | 1.62 | 1.37 | 2.55 | 4.97 | 4.02 | 1.57 |
| | Barley (Total) | Correlation | +.528 ±.071 | −.047 ±.099 | −.265 ±.092 | −.146 ±.097 | −.249 ±.093 | −.321 ±.089 | −.320 ±.089 | +.033 ±.099 |
| | | r/Er | 7.36 | .47 | 2.87 | 1.50 | 2.67 | 3.60 | 3.59 | .33 |
| 1922 | Alfalfa III | Correlation | | +.027 ±.099 | +.069 ±.098 | +.049 ±.099 | +.074 ±.098 | +.147 ±.097 | +.117 ±.098 | +.185 ±.096 |
| | | r/Er | | .28 | .69 | .50 | .75 | 1.51 | 1.19 | 1.92 |
| 1923 | Alfalfa I | Correlation | +.027 ±.099 | | +.457 ±.078 | +.926 ±.014 | +.717 ±.048 | +.155 ±.097 | +.601 ±.063 | +.175 ±.096 |
| | | r/Er | .28 | | 5.81 | 66.2 | 14.9 | 1.60 | 9.49 | 1.81 |
| | Alfalfa III | Correlation | +.069 ±.098 | +.457 ±.078 | | +.757 ±.042 | +.710 ±.049 | +.410 ±.082 | +.703 ±.050 | −.119 ±.098 |
| | | r/Er | .69 | 5.81 | | 17.9 | 14.4 | 4.97 | 14.0 | 1.21 |
| | Alfalfa I and III | Correlation | +.049 ±.099 | +.926 ±.014 | +.757 ±.042 | | +.826 ±.031 | +.287 ±.091 | +.738 ±.045 | +.078 ±.098 |
| | | r/Er | .50 | 66.2 | 17.9 | | 26.2 | 3.15 | 16.4 | .79 |
| | Alfalfa II | Correlation | +.074 ±.098 | +.717 ±.048 | +.710 ±.049 | +.826 ±.031 | | +.424 ±.081 | +.925 ±.014 | +.129 ±.097 |
| | | r/Er | .75 | 14.9 | 14.4 | 26.2 | | 5.20 | 65.2 | 1.31 |
| 1924 | Alfalfa III | Correlation | +.147 ±.097 | +.155 ±.097 | +.410 ±.082 | +.287 ±.091 | +.424 ±.081 | | +.735 ±.045 | −.073 ±.098 |
| | | r/Er | 1.51 | 1.60 | 4.97 | 3.15 | 5.20 | | 16.1 | .74 |
| | Alfalfa II and III | Correlation | +.117 ±.098 | +.601 ±.063 | +.703 ±.050 | +.738 ±.045 | +.925 ±.014 | +.735 ±.045 | | +.065 ±.099 |
| | | r/Er | 1.19 | 9.49 | 14.0 | 16.4 | 65.2 | 16.1 | | .66 |
| 1925 | Silage corn | Correlation | +.185 ±.096 | +.175 ±.096 | −.119 ±.098 | +.078 ±.098 | +.129 ±.097 | −.073 ±.098 | +.065 ±.099 | |
| | | r/Er | 1.92 | 1.81 | 1.21 | .79 | 1.31 | .74 | .66 | |

In Figures 2 to 4 the correlation between each of the crop yields and all of the other crop yields [3] is indicated in one of the panels. The crop considered as the primary variable is shown on each panel. The crop yields considered as the secondary variables are shown at the bottom of each figure. In each panel a heavy bar represents

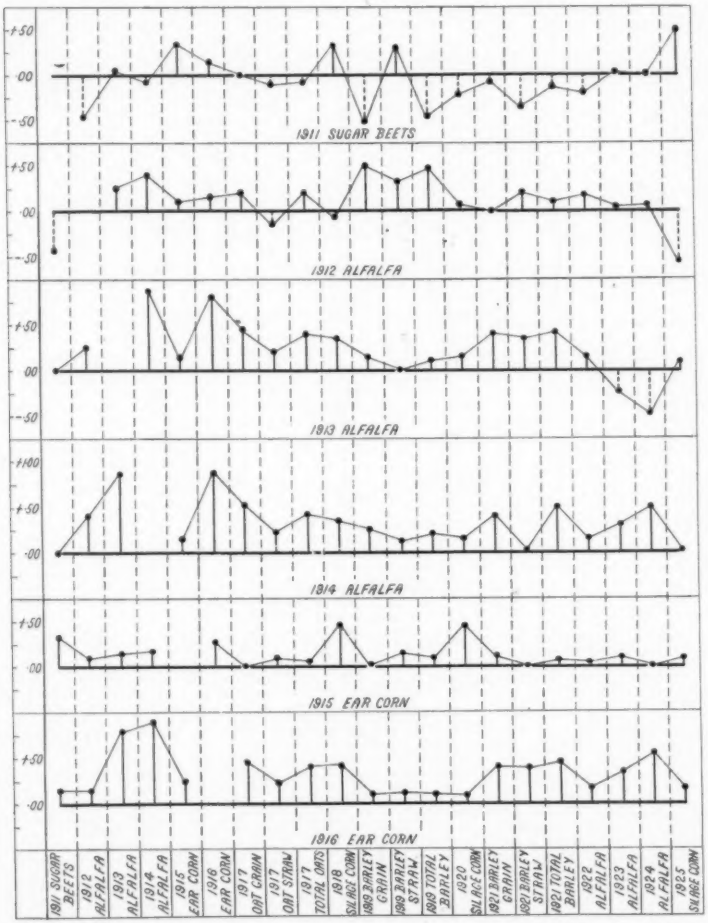


FIG. 2.—Magnitudes of correlations between the yields of various crops as grown in the uniform-cropping experiments at the Huntley Field Station, 1911–1916

the zero correlation which should be found if there were no permanency of the differences in the crop-producing capacity of the several plots of the field. Positive correlations are represented by the magnitudes of the deviations above this line, and negative correlations

[3] In the case of the alfalfa crops the correlations for total yield only are represented, not those for individual cuttings.

are represented by the deviations below this line, as shown on the scale of ordinates.

The results obtained with respect to individual crops merit detailed consideration. The correlations for the yield of sugar beets in 1911 with the 20 subsequent crop yields show large irregularities. Prac-

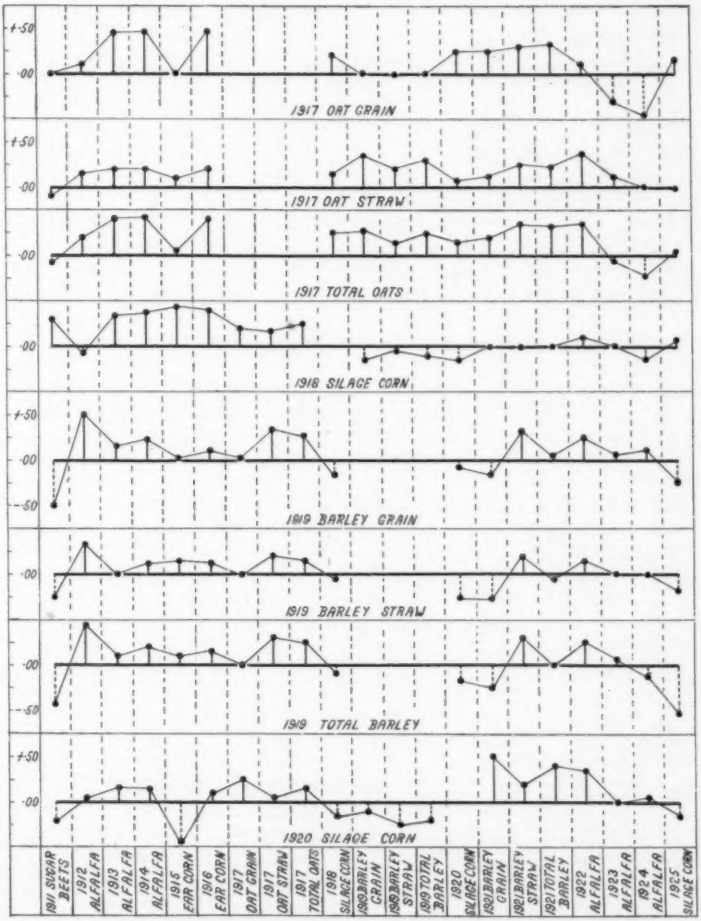


FIG. 3.—Magnitudes of correlations between the yields of various crops as grown in the uniform-cropping experiments at the Huntley Field Station, 1917–1920

tically as many negative as positive correlations are seen. Taking the series as a whole, it appears that variations in the factors influencing the yield of sugar beets on these plots had no relationship to the yields of subsequent crops.

In 1912 the first of the crops of the first stand of alfalfa was harvested. For all of these crops (1912, 1913, 1914) the correlations

with antecedent crops and with practically all subsequent crops are positive in sign. Thus the original heterogeneity factors of the fields, or those introduced by differences in stand of alfalfa, persisted practically throughout the period of cultivation of this field. An exception is noted in the case of the correlation with the 1911 crop

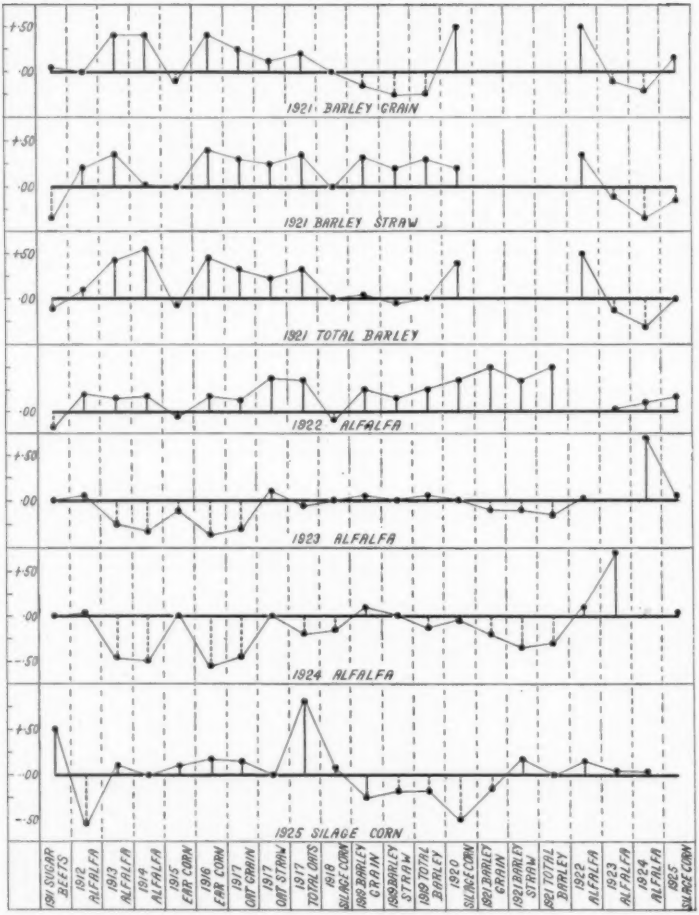


FIG. 4.—Magnitudes of correlations between the yields of various crops as grown in the uniform-cropping experiments at the Huntley Field Station, 1921–1925

of sugar beets and with the 1923 and 1924 crops of alfalfa and the 1925 crop of silage corn. The sensibly zero or even negative correlation with the crops of alfalfa grown 10 years later indicates that plots which were particularly suited for alfalfa at one period were not necessarily especially adapted to this crop at a subsequent period.

Consideration of the underlying causes of these differences in correlation is reserved until the present review of the results for the several crops is completed.

The correlations between the two crops of ear corn (1915 and 1916) and the other yields considered are generally positive. Those for 1916 are sensibly higher than those for the earlier year. This point has been considered in some detail in the former paper (7). Differences in the conditions of the soil, original or induced by agricultural technic and expressed in variations in the yield of ear corn, are therefore reflected in the yields of the other crops throughout the period of the investigation.

The three measures of oat yield in the crop grown in 1917 show a generally positive correlation with other yields except for the sugar-beet crop of 1911 and the alfalfa crops of 1923 and 1924 and the silage-corn yield of 1925. These will be considered later.

Correlations for the silage-corn yields (1918) with preceding crops are generally positive, the only exception being that for the 1912 crop of alfalfa. The relationship between the yields of this crop of silage corn and the yields of subsequent crops are, however, sensibly zero.

The three measures of the yield of barley as grown in 1919 are in general positively correlated with the yields of other crops. It is, however, interesting to note that they show a slightly negative correlation with the yield of silage corn of the preceding year (1918), of the year immediately following (1920), and of the sixth subsequent year (1925).

The 1920 crop of silage corn shows positive correlations with 12 of the other crops and negative correlations with 8 of the other crops. It shows a positive correlation with the three barley yields of the following year. This indicates that there is no necessary inverse relationship between the capacity of these plots for producing barley and corn. Turning to the three yields which furnish a measure of the capacity of these plots for barley production as grown in 1921, it is noted that in general the correlations are positive. The conspicuous exceptions are seen in the constants for alfalfa as grown in 1923 and 1924. These will be considered presently.

It should be noted that the correlations between barley as grown in 1921 and the first available test of silage corn, 1918, are sensibly zero. The correlations for the 1925 crop of silage corn are also sensibly zero. Those for the 1920 crop of silage corn, however, are more substantial positive magnitudes. The correlations with the 1915 crop of ear corn are sensibly zero, but those with the 1916 crop of ear corn are more substantial positive values. Taking these results as a whole, they indicate that there is no definitely demonstrated evidence for a negative correlation between the capacities of the plots for the production of barley and corn.

Beginning with 1922, the area was again put into alfalfa. The alfalfa yield of 1922 shows in general a positive correlation with the yields of preceding crops, but practically no correlation with the following two years' records of alfalfa or with the third subsequent year's yield of silage corn.

In the preceding investigation (7) the yields of alfalfa were found to be highly correlated with the yields of other crops. Since alfalfa occurred early in the rotation adopted in these experiments, it is

possible that differences in the yields of alfalfa influenced subsequent crops by the introduction of differences in the nitrogen content or tilth of the soil of the various plots. The present results for alfalfa are more irregular. The cutting of 1922 shows in general a positive correlation with other yields. The record for 1923 shows a sensibly zero correlation with the harvest of oat straw of 1917 to alfalfa yield of 1922. The correlation with the 1924 crop of alfalfa is high, as might be expected from the fact that both of them are influenced by the stand obtained. The correlation with the 1925 crop of silage corn is sensibly zero. The correlations with the preceding crops from the 1913 yields of alfalfa to the 1917 yields of oat grain are actually negative. Similar results are found for the 1924 crop of alfalfa. It is important to note, however, that these correlations are based on the total yields of alfalfa for these years. In both of these years one cutting (the second cutting in 1923 and the first cutting in 1924) of alfalfa was not weighed by plots. Thus, it might be suggested that because of the incompleteness of the data these yields may be inaccurate as a measure of the actual annual producing capacity of the fields. If one deals with only the first cutting in 1923, however, it is noted that only 12 of the 28 correlations are positive. If one deals with the third cutting in 1923, it is found that only 11 of the 28 correlations are positive. Similarly, if one considers only the second cutting of 1924, it appears that only 10 of the 28 correlations are positive. For the third cutting of 1924 only 8 of the 28 correlations are positive. Thus, there is clear evidence that for the individual cuttings of alfalfa there is a sensibly zero or even a negative correlation between the yields of 1923 and 1924 and those of other crops grown previously on these areas.

Turning to the final crop, silage corn as grown in 1925, it is clear that the correlations are about evenly distributed between positive and negative values.

## DISCUSSION OF RESULTS

In an earlier investigation (*7*), based on the results of cultures grown from 1911 to 1919, inclusive, a high preponderance of positive correlations (many of which were clearly significant in comparison with their individual probable errors) was found between the yields of a series of plots in one year and the yields of these plots in another year. These relationships between yields were found to hold whether the crop grown was the same or different in the two years. Such results indicate clearly a relatively high permanence of the differences in the plots of the experimental field.

These findings have been confirmed by many of the constants deduced for the first time in the present investigation involving crops grown for the years 1920 to 1925. In a number of cases, however, negative correlations between the yields of crops grown on the same plots in different years have been demonstrated. This is conspicuous in the case of the correlations for alfalfa grown near the end of the experimental period.

To provide a general survey of the whole series of constants, Table 4 has been prepared. This shows the distribution of the magnitudes of the correlations between each of the crops grown and all of the other crops with the exception of crops which have a direct organic relation to the several crops listed. These have been omitted. For example, in dealing with alfalfa the correlations between the different

cuttings in one and the same year have been omitted. Similarly, the correlation between grain and straw and between either grain or straw and total yield have been omitted in the case of the cereals.

TABLE 4.—*Magnitudes of correlation coefficients measuring the relationship of crop yields on plots at Huntley, Mont., in different years*

| Year | Crop | −0.650 to −0.551 | −0.550 to −0.451 | −0.450 to −0.351 | −0.350 to −0.251 | −0.250 to −0.151 | −0.150 to −0.051 | −0.050 to +0.050 | +0.051 to +0.150 | +0.151 to +0.250 | +0.251 to +0.350 | +0.351 to +0.450 | +0.451 to +0.550 | +0.551 to +0.650 | +0.651 to +0.750 | +0.751 to +0.850 | +0.851 to +0.950 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1911 | Sugar beets | | 2 | 2 | 1 | 3 | 6 | 9 | 3 | 1 | 2 | | 1 | | | | |
| 1912 | Alfalfa | 1 | 1 | | | | 1 | 3 | 7 | 9 | 2 | 4 | 2 | | | | |
| 1913 | Alfalfa I | 1 | | 1 | 1 | 1 | 2 | 3 | 5 | 3 | 1 | 2 | 3 | 2 | | 3 | |
| | Alfalfa II | | | 2 | 3 | 1 | | 2 | 2 | 8 | 1 | 2 | 1 | 2 | 3 | 1 | |
| | Alfalfa I and II | | 2 | 1 | 2 | 1 | | 2 | 2 | 6 | 1 | 4 | 1 | | 1 | 2 | 3 |
| 1914 | Alfalfa I | | 2 | 2 | 1 | 1 | | 1 | 2 | 4 | 3 | 3 | 2 | 2 | | 2 | 1 |
| | Alfalfa II | | 2 | 3 | 1 | 1 | | 2 | 3 | 3 | 3 | 3 | 1 | 1 | 1 | 2 | |
| | Alfalfa I and II | 2 | 1 | 2 | 1 | | 1 | | 2 | 6 | 1 | 3 | 3 | | 1 | 1 | 2 |
| | Alfalfa III | | | 1 | 1 | 2 | 3 | 2 | 4 | 1 | 5 | 2 | 1 | 2 | 2 | | |
| | Alfalfa I to III | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 6 | | 4 | 2 | | | 2 | 2 |
| 1915 | Ear corn | | 1 | | | | 6 | 8 | 6 | 4 | 4 | | 1 | | | | |
| 1916 | do | 2 | 1 | 2 | 1 | | | | 4 | 5 | 1 | 4 | 2 | 1 | 2 | 3 | 2 |
| 1917 | Oats: Grain | | 1 | 3 | 1 | 1 | | 5 | 1 | 3 | 4 | 4 | 5 | | | | |
| | Oats: Straw | | | | | | 1 | 4 | 7 | 10 | 4 | 2 | | | | | |
| | Oats: Total | | | | | 3 | 3 | 1 | 2 | 4 | 6 | 9 | | | | | |
| 1918 | Silage corn | | | | | 4 | 6 | 4 | 2 | 6 | 3 | 2 | 2 | 1 | | | |
| 1919 | Barley: Grain | | 1 | | 1 | 2 | 1 | 4 | 7 | 4 | 6 | 1 | 1 | | | | |
| | Barley: Straw | | | | 3 | 1 | 3 | 7 | 7 | 6 | 1 | | | | | | |
| | Barley: Total | | 2 | 1 | 1 | 1 | 2 | 4 | 7 | 5 | 4 | | 1 | | | | |
| 1920 | Silage corn | | 1 | | 1 | 4 | 4 | 2 | 6 | 8 | 1 | 2 | 1 | | | | |
| 1921 | Barley: Grain | | | | 3 | 4 | 2 | 3 | 2 | 4 | 2 | 4 | 4 | | | | |
| | Barley: Straw | | | 2 | 1 | 3 | 2 | 3 | | 4 | 5 | 6 | 2 | | | | |
| | Barley: Total | | | | 3 | 1 | 4 | 4 | 2 | 2 | 3 | 3 | 4 | 2 | | | |
| 1922 | Alfalfa III | | | | | 1 | 2 | 2 | 8 | 8 | 3 | 4 | 2 | | | | |
| 1923 | Alfalfa I | | | | 3 | 5 | 4 | 9 | 3 | 2 | | | | 1 | 1 | | |
| | Alfalfa III | | 1 | 5 | 4 | 2 | 7 | | 4 | 2 | | 1 | | | 2 | | |
| | Alfalfa I and III | | | 3 | 5 | 1 | 7 | 5 | 4 | | 1 | | | | 1 | 1 | |
| 1924 | Alfalfa II | | 1 | 7 | 1 | 5 | 2 | 3 | 6 | | | | | | 2 | 1 | |
| | Alfalfa III | 4 | 4 | 3 | 2 | 3 | 2 | 5 | 2 | 1 | 1 | 1 | | | | | |
| | Alfalfa II and III | 2 | 5 | 3 | 2 | 3 | 2 | 4 | 4 | | | | | 1 | 2 | | |
| 1925 | Silage corn | 1 | 1 | | 1 | 3 | 2 | 5 | 11 | 5 | | | 1 | | | | |
| | Total | 14 | 30 | 45 | 45 | 58 | 76 | 108 | 126 | 130 | 68 | 70 | 43 | 15 | 18 | 18 | 10 |

The frequency distributions of correlation coefficients in this table show a rather wide range of both positive and negative values. These have been considered in detail on the basis of the three diagrams constituting Figures 2, 3, and 4, and nothing further need be said for the moment in regard to the relationships for the individual crops.

Taking the materials as a whole, it appears that 108 of the coefficients fall essentially in the class of zero correlations—i. e., of −0.05 to +0.05.[4] The distribution of positive and negative coefficients on the two sides of this arbitrarily limited (zero) value is very unlike. Thus 76 constants fall between −0.051 and −0.150, whereas 126 fall between the limits +0.051 and +0.150. Similarly, only 58 coefficients fall between the limits of −0.151 and −0.250, whereas more than twice this number (130) fall between +0.151 and +0.250. Again, only 45 coefficients fall between the limits of −0.251 and −0.350, whereas 68 fall between +0.251 and +0.350. Finally, only 89 fall below the limit −0.350, whereas 174 fall above +0.350.

Thus the results indicate clearly the great preponderance of positive correlations for the series as a whole. Notwithstanding this fact, the figures just given show that many fairly substantial negative

[4] This range is probably too narrow for the zero class of coefficients based on samples of only 46 plots, but it forms a convenient unit for present purposes

coefficients occur. It is now necessary to estimate the significance of these when considered in relation to their probable errors.

The ratios of these coefficients to their probable errors, as computed by the usual formula, appear in Table 5. These ratios are arranged in class intervals of 2.50. Of these values, 299 positive coefficients fall between +0 and +2.50, whereas 169 fall between the limits of −0 and −2.50. A total of 136 coefficients fall between +2.51 and +5.00, whereas 88 coefficients fall between −2.51 and −5.00. Finally, 128 positive coefficients are more than five times as large as their probable errors, whereas only 54 negative coefficients are more than five times as large as their probable errors.

These results indicate clearly that, while there is a preponderance of positive correlations in this series and while the positive coefficients taken as a class have a greater probability of significance, certain of the negative coefficients are statistically significant in comparison with their probable errors. It appears, therefore, that in general plots which yield better in certain individual years will give better results throughout a long period of time, but that under certain conditions they may actually give yields significantly below the average.

Having demonstrated that both positive and negative correlations occur as measures of interrelationship between the crop yields of the 15 years over which these experimental cultures have extended, it still remains to analyze the series of coefficients more minutely with a view to determining, if possible, what the reasons for the apparent inconsistencies may be.

TABLE 5.—*Ratios of correlation coefficients measuring the interrelationship of yields to their probable errors for crops grown in different years on plots at Huntley, Mont.*

| Year | Crop | −7.51 to −10.00 | −5.01 to −7.50 | −2.51 to −5.00 | −0 to −2.50 | +0 to +2.50 | +2.51 to +5.00 | +5.01 to +7.50 | +7.51 to +12.50 | +12.51 to +17.50 | +17.51 to +25.00 | +25.01 to +37.50 | +37.51 to +42.50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1911 | Sugar beets | 1 | 2 | 3 | 13 | 8 | 2 | 1 | ---- | ---- | ---- | ---- | ---- |
| 1912 | Alfalfa | 1 | 1 | 0 | 3 | 15 | 6 | 4 | ---- | ---- | ---- | ---- | ---- |
| 1913 | Alfalfa I | 1 | 1 | 1 | 4 | 9 | 3 | 4 | 2 | ---- | 2 | 1 | ---- |
| | Alfalfa II | ---- | ---- | 5 | 1 | 11 | 3 | 2 | 2 | 4 | ---- | ---- | ---- |
| | Alfalfa I and II | ---- | 2 | 3 | 1 | 9 | 5 | 2 | 1 | ---- | 2 | 1 | 2 |
| 1914 | Alfalfa I | 1 | 1 | 3 | 2 | 6 | 5 | 3 | 2 | ---- | 1 | 2 | ---- |
| | Alfalfa II | 1 | 2 | 3 | 1 | 7 | 7 | 1 | 1 | 1 | 2 | ---- | ---- |
| | Alfalfa I and II | 2 | 1 | 3 | 1 | 6 | 4 | 5 | ---- | 1 | 1 | 1 | 1 |
| | Alfalfa III | ---- | ---- | 2 | 6 | 5 | 6 | 3 | 3 | 1 | ---- | ---- | ---- |
| | Alfalfa I to III | 1 | 1 | 4 | 1 | 7 | 4 | 3 | 1 | 1 | 1 | ---- | 2 |
| 1915 | Ear corn | ---- | 1 | ---- | 9 | 15 | 4 | 1 | ---- | ---- | ---- | ---- | ---- |
| 1916 | ....do | 2 | 2 | 2 | ---- | 9 | 4 | 3 | 1 | 2 | 3 | 1 | 1 |
| 1917 | Oats—Grain | ---- | 2 | 3 | 4 | 6 | 5 | 7 | 1 | ---- | ---- | ---- | ---- |
| | Oats—Straw | ---- | ---- | ---- | 4 | 16 | 8 | ---- | ---- | ---- | ---- | ---- | ---- |
| | Oats—Total | ---- | ---- | ---- | 7 | 5 | 14 | 2 | ---- | ---- | ---- | ---- | ---- |
| 1918 | Silage corn | ---- | ---- | ---- | 10 | 10 | 6 | 3 | 1 | ---- | ---- | ---- | ---- |
| 1919 | Barley—Grain | 1 | ---- | 1 | 4 | 13 | 8 | 1 | ---- | ---- | ---- | ---- | ---- |
| | Barley—Straw | ---- | ---- | 3 | 7 | 17 | 1 | ---- | ---- | ---- | ---- | ---- | ---- |
| | Barley—Total | 2 | 1 | 1 | 4 | 14 | 5 | 1 | ---- | ---- | ---- | ---- | ---- |
| 1920 | Silage corn | ---- | 1 | 2 | 8 | 15 | 2 | 2 | ---- | ---- | ---- | ---- | ---- |
| 1921 | Barley—Grain | ---- | ---- | 3 | 6 | 7 | 7 | 4 | 1 | ---- | ---- | ---- | ---- |
| | Barley—Straw | ---- | ---- | 4 | 5 | 6 | 10 | 3 | ---- | ---- | ---- | ---- | ---- |
| | Barley—Total | ---- | ---- | 4 | 5 | 5 | 6 | 7 | 1 | ---- | ---- | ---- | ---- |
| 1922 | Alfalfa III | ---- | ---- | ---- | 3 | 18 | 7 | 1 | 1 | ---- | ---- | ---- | ---- |
| 1923 | Alfalfa I | ---- | ---- | 4 | 14 | 8 | ---- | ---- | 1 | 1 | ---- | ---- | ---- |
| | Alfalfa III | ---- | 3 | 7 | 9 | 6 | 1 | ---- | ---- | 2 | ---- | ---- | ---- |
| | Alfalfa I and III | ---- | ---- | 8 | 9 | 8 | 1 | ---- | ---- | 1 | ---- | 1 | ---- |
| | Alfalfa II | ---- | 1 | 10 | 7 | 7 | ---- | ---- | ---- | 2 | ---- | 1 | ---- |
| 1924 | Alfalfa III | 7 | 2 | 4 | 7 | 6 | 2 | ---- | ---- | ---- | ---- | ---- | ---- |
| | Alfalfa II and III | 2 | 6 | 4 | 7 | 6 | ---- | ---- | 1 | 2 | ---- | ---- | ---- |
| 1925 | Silage corn | 2 | ---- | 1 | 7 | 19 | ---- | 1 | ---- | ---- | ---- | ---- | ---- |
| | Total | 24 | 30 | 88 | 169 | 299 | 136 | 64 | 20 | 18 | 12 | 8 | 6 |

The foregoing relationships as indicated by the average values of the correlations for each of the crops grown will be first considered.

In computing these averages the constants may be divided into two groups. The first comprises correlations between each crop and every other crop grown for the period 1911 to 1919, inclusive, and is therefore identical with that for the period covered by the first investigation (*7*). The second comprises all correlations for the period 1920 to 1925, inclusive.

The averages and the number of correlations on which these are based are given in columns 3 to 6 of Table 6. The averages for the whole period considered, 1911 to 1925, are given in columns 7 and 8.

TABLE 6.—*Averages of correlations measuring the interrelationship of yields for crops grown on plots at Huntley, Mont., in different years*

| Year | Crop | 1911–1919 | | 1920–1925 | | 1911–1925 | | 1911–1921 | | 1923–1924 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *N* | *r* | *N* | *r* | *N* | *r* | *N* | *r* | *N* | *r* |
| 1911 | Sugar beets | 18 | −0.0772 | 12 | −0.0286 | 30 | −0.0578 | 22 | −0.0927 | 6 | −0.0013 |
| 1912 | Alfalfa | 18 | +.2421 | 12 | +.0282 | 30 | +.1566 | 22 | +.2170 | 6 | +.0499 |
| 1913 | Alfalfa I | 16 | +.3456 | 12 | +.0009 | 28 | +.1978 | 20 | +.3525 | 6 | −.2832 |
| | Alfalfa II | 16 | +.4026 | 12 | −.0606 | 28 | +.2041 | 20 | +.3614 | 6 | −.3106 |
| | Alfalfa I and II | 16 | +.4412 | 12 | −.0345 | 28 | +.2373 | 20 | +.4224 | 6 | −.3480 |
| 1914 | Alfalfa I | 14 | +.4012 | 12 | −.0220 | 26 | +.2059 | 18 | +.4108 | 6 | −.4015 |
| | Alfalfa II | 14 | +.3542 | 12 | −.0920 | 26 | +.1483 | 18 | +.3454 | 6 | −.4167 |
| | Alfalfa I and II | 14 | +.4068 | 12 | −.0639 | 26 | +.1896 | 18 | +.4066 | 6 | −.4427 |
| | Alfalfa III | 14 | +.3661 | 12 | −.0200 | 26 | +.1879 | 18 | +.3426 | 6 | −.2187 |
| | Alfalfa I to III | 14 | +.4276 | 12 | −.0917 | 26 | +.1879 | 18 | +.3961 | 6 | −.4100 |
| 1915 | Ear corn | 18 | +.1673 | 12 | −.0865 | 30 | +.0658 | 22 | +.1031 | 6 | −.0573 |
| 1916 | do | 18 | +.4863 | 12 | −.0765 | 30 | +.2612 | 22 | +.4602 | 6 | −.4458 |
| 1917 | Oats: Grain | 16 | +.2893 | 12 | −.0547 | 28 | +.1419 | 20 | +.2913 | 6 | −.3630 |
| | Oats: Straw | 16 | +.2013 | 12 | +.1244 | 28 | +.1683 | 20 | +.1983 | 6 | +.0632 |
| | Oats: Total | 16 | +.2925 | 12 | +.0659 | 28 | +.1954 | 20 | +.2912 | 6 | −.1310 |
| 1918 | Silage corn | 18 | +.2259 | 12 | −.0467 | 30 | +.1169 | 22 | +.1813 | 6 | −.0751 |
| 1919 | Barley: Grain | 16 | +.1405 | 12 | +.0591 | 28 | +.1056 | 20 | +.1200 | 6 | +.0929 |
| | Barley: Straw | 16 | +.0863 | 12 | −.0229 | 28 | +.0395 | 20 | +.0485 | 6 | +.0237 |
| | Barley: Total | 16 | +.1264 | 12 | −.0629 | 28 | +.0453 | 20 | +.0950 | 6 | −.0593 |
| 1920 | Silage corn | 19 | +.0285 | 11 | +.1010 | 30 | +.0551 | 22 | +.0780 | 6 | −.0452 |
| 1921 | Barley: Grain | 19 | +.1736 | 9 | +.0322 | 28 | +.1282 | 20 | +.1916 | 6 | −.1618 |
| | Barley: Straw | 19 | +.2549 | 9 | −.1108 | 28 | +.1373 | 20 | +.2525 | 6 | −.2374 |
| | Barley: Total | 19 | +.2614 | 9 | −.0394 | 28 | +.1647 | 20 | +.2701 | 6 | −.2252 |
| 1922 | Alfalfa III | 19 | +.1535 | 11 | +.2258 | 30 | +.1800 | 23 | +.2056 | ---- | ---- |
| 1923 | Alfalfa I | 19 | −.1008 | 9 | +.1736 | 28 | −.0126 | 23 | −.0882 | ---- | ---- |
| | Alfalfa III | 19 | −.1671 | 9 | +.1052 | 28 | −.0796 | 23 | −.1740 | ---- | ---- |
| | Alfalfa I and III | 19 | −.1584 | 9 | +.1718 | 28 | −.0522 | 23 | −.1497 | ---- | ---- |
| 1924 | Alfalfa II | 19 | −.1894 | 9 | +.1813 | 28 | −.0702 | 23 | −.1924 | ---- | ---- |
| | Alfalfa III | 19 | −.3065 | 9 | +.0125 | 28 | −.2039 | 23 | −.2886 | ---- | ---- |
| | Alfalfa II and III | 19 | −.2697 | 9 | +.1409 | 28 | −.1377 | 23 | −.2645 | ---- | ---- |
| 1925 | Silage corn | 19 | +.0024 | 11 | +.0315 | 30 | +.0131 | 23 | −.0021 | 6 | +.0425 |

The number of constants averaged is larger than the number of years of experimentation because of the fact that more than one yield is available for certain of the crops (small grains and alfalfa). The correlations considered, however, have not involved relationships between two different measures of one and the same crop grown in a given year. Thus, correlations between yields of grain and straw, or between grain or straw and total yield, in the cereal crop of a given year have been excluded. Similarly, correlations between the different cuttings of alfalfa in the same year have been omitted.

In considering the values in this table it may be noted that, with the exception of the 1911 tonnage of sugar beets and the 1923 and 1924 cuttings of alfalfa, all of the average correlations for the period 1911 to 1919 are positive.

Before turning to a more general discussion of these averages, it may be noted that the yields of the single crop of sugar beets grown in 1911 have shown anomalies in correlation from the first. Unfortunately, no very precise information is available concerning the exact conditions prevailing during the first year of these experiments. Prior to the growing of sugar beets in 1911 the field had not been cropped uniformly. These antecedent differences of treatment may have led to diversities in the 1911 yields, but this is not at all certain. The writers are inclined to the view that owing to necessary difficulties in initiating these experiments in 1911 the preparation and leveling of the land may not have been as well done as in later years when the details could be cared for by men more experienced in the work. In view of these uncertainties, the data for sugar beets must be essentially disregarded in the interpretation of the results of the experiments as a whole. They have been included in the averages in order to avoid introducing any question of bias in selecting the constants on which conclusions are based. The averages of the correlations of each of the yields for the 15-year period 1911 to 1925 with the yields for 1920 to 1925 (columns 5 and 6 of Table 6) are by no means so consistent as those for the period 1911 to 1919. Of the 31 averages for 1920 to 1925, 15 are positive and 16 are negative.

An examination of the averages for the whole period of 15 years (columns 7 and 8 of Table 6) furnishes a suggestion as to the possible source of the discrepancy between the first two series of averages.

The mean correlations for the period 1911 to 1925 are positive for each of the 31 crop yields considered with the exception of the one crop of sugar beets and the cuttings of alfalfa for 1923 and 1924. This suggests that the yield of alfalfa for 1923 and 1924 may be a primary source of the frequent negative values in the series of averages for the period 1920 to 1925.

Turning back to Table 4, which gives the distribution of the various interannual correlation coefficients with regard to signs for each of the crop yields, it is noted that of the 268 correlation coefficients which are more negative than $-0.050$ (i. e., those which fall to the left of the class which is considered to represent essentially zero correlation), 101 occur with the crops of alfalfa of 1923 and 1924.

Two new series of averages, therefore, have been determined. The first represents all crop yields from the beginning of the experiment to 1921, or until the time when the area was again put into alfalfa. The second represents the average correlation of the yields of alfalfa in 1923 and 1924 with each of the other crops (excepting 1922, 1923, and 1924 alfalfa) grown during the period 1911 to 1924.

The first of these two series of averages, presented in columns 9 and 10 of Table 6, are positive values except for the sugar-beet yield of 1911 (which has proved anomalous in practically all cases), the alfalfa yields of 1923 and 1924, and the yield of silage corn in 1925, which is sensibly zero.

The second series of averages (columns 11 and 12 of Table 6) shows 19 negative averages as compared with only 5 which are positive.

In the earlier investigation (7) emphasis was placed on certain peculiarities of the correlation for alfalfa, particularly those associated with repeated cuttings and with the after effect of the alfalfa crop.

In this connection it is worth while to lay side by side (*a*) the correlations between the yields of alfalfa as given in the first stand (1912–1914) and the yield of subsequent crops up to the times of reseeding to alfalfa (1915–1921) and (*b*) the correlation between the yields of alfalfa grown in the second stand (1922–1924) and the yields of antecedent crops back to the previous stand of alfalfa (1915–1921). Since, as already noted (7), it is quite reasonable to assume that in a crop harvested more than once a year thickness of stand and variation in the size of the individual plants will have a large influence on the yields of the different plots in the same year, the averages of the correlations between the different cuttings of the same year as well as of those between single cuttings and total cuttings in the different years of the same stand have been placed beside the above averages. The results for the correlations of alfalfa yields with the yields of subsequent crops are given in Table 7. Those for the correlation of alfalfa with the yields of antecedent crops are set forth in Table 8.

TABLE 7.—*Comparison of the averages of the correlations of alfalfa in the cuttings of 1912–1914 (a) with the yields of subsequent crops of other kinds and (b) with cuttings of alfalfa in the three different years of the same stand*

[Compare Table 8]

| Year | Alfalfa crops of first stand | Average correlation with— | | | | Difference |
|---|---|---|---|---|---|---|
| | | (*a*) Other crops in 1915–1921 | | (*b*) Alfalfa yields in 1912–1914 | | |
| | | *N* | *r* | *N* | *r* | |
| 1912 | One cutting | 13 | +0.1986 | 8 | +0.3305 | +0.1319 |
| 1913 | First cutting | 13 | +.2565 | 6 | +.6107 | +.3542 |
| | Second cutting | 13 | +.2802 | 6 | +.6038 | +.3236 |
| | First and second cuttings | 13 | +.3146 | 6 | +.7202 | +.4056 |
| 1914 | First cutting | 13 | +.3654 | 4 | +.6660 | +.3006 |
| | Second cutting | 13 | +.2971 | 4 | +.6293 | +.3322 |
| | First and second cuttings | 13 | +.3559 | 4 | +.6988 | +.3439 |
| | Third cutting | 13 | +.3146 | 4 | +.5243 | +.2097 |
| | First, second, and third cuttings | 13 | +.3377 | 4 | +.7063 | +.3686 |

TABLE 8.—*Comparison of the averages of the correlations of alfalfa in the cuttings of 1922–1924 (a) with the yields of antecedent crops of other kinds and (b) with cuttings of alfalfa in the three different years of the same stand*

[Compare Table 7]

| Year | Alfalfa crops of second stand | Average correlation with— | | | | Difference |
|---|---|---|---|---|---|---|
| | | (*a*) Other crops in 1915–1921 | | (*b*) Alfalfa yields in 1922–1924 | | |
| | | *N* | *r* | *N* | *r* | |
| 1922 | One cutting | 13 | +0.2661 | 6 | +0.0810 | −0.1851 |
| 1923 | First cutting | 13 | −.0421 | 4 | +.3758 | +.4179 |
| | Third cutting | 13 | −.1052 | 4 | +.4738 | +.5790 |
| | First and third cuttings | 13 | −.0954 | 4 | +.4758 | +.5712 |
| 1924 | Second cutting | 13 | −.1342 | 4 | +.5820 | +.7162 |
| | Third cutting | 13 | −.2031 | 4 | +.2508 | +.4539 |
| | First, second, and third cuttings | 13 | −.1853 | 4 | +.5405 | +.7258 |

Table 7 shows that the averages of the correlations between the various yields for the stand of alfalfa which occupied the land from 1912 to 1914 and the yields of subsequent crops of other kinds for the period 1915 to 1921, inclusive, are without exception positive values which range from +0.20 to +0.37. These averages are much lower than those which are found for the correlations between the yields of alfalfa in the different years of this stand. The magnitudes of the differences between the correlations between the yields of alfalfa and alfalfa and between alfalfa and other crops are clearly shown by the entries in the final column.

Turning to the averages of the correlations between the yields of alfalfa for the stand of 1922 to 1924 and the yields of antecedent crops of a different kind for the period 1915 to 1921, as shown in Table 8, a very different result is noted. These averages are negative with the exception of that for the single cutting of 1922. The averages of the correlations between the various cuttings of alfalfa in the three different years of the period 1922 to 1924 are of material positive value.

The final column shows the difference between the correlations for (*a*) the alfalfa cuttings of the stand and the yields of other crops and for (*b*) the yields of alfalfa.

A comparison of the averages in these two tables shows clearly (1) that the correlations between the yields of alfalfa in the different years are high in the case of both stands, which were separated by a period of seven years, but (2) that the correlations of subsequent crops with the stand of 1912 to 1914 are on an average positive, whereas those for antecedent crops with the stand for 1922 to 1924 are on an average negative.

It is evident that something connected with the stand or rankness of growth of the alfalfa in the latter period has been of primary importance in modifying the characteristics of the plots (as measured in terms of crop yield) as they prevailed during the first 10 years of the experiments.

In connection with the problem of changes in the heterogeneity of the field the realtionships between the various yields of alfalfa in the first and second periods are of particular interest.

The individual constants required can be obtained from data given in Table 3, pages 22 and 23.

The 63 correlation coefficients for the relationship between the two different stands of alfalfa designated in Table 3 are prevailingly negative in sign. Only 13 values are positive, as compared with 50 which are negative. Of these 13 positive values, 9 are found in the correlations between the yield of alfalfa in 1922 and the yields of alfalfa in 1912, 1913, and 1914. Of the 54 correlations between cuttings of 1923 and 1924 and cuttings of 1912, 1913, and 1914, only 4 are positive.

TABLE 9.—*Averages of correlations between cuttings of alfalfa in the first and the second stands*

[Original constants are given in Table 3]

| Year | Alfalfa crops | N | Average correlation with alfalfa yields |
|---|---|---|---|
| | | | *1922–1924* |
| 1912 | One cutting | 7 | +0.0707 |
| 1913 | First cutting | 7 | −.2342 |
| | Second cutting | 7 | −.2368 |
| | First and second cuttings | 7 | −.2762 |
| 1914 | First cutting | 7 | −.3020 |
| | Second cutting | 7 | −.3403 |
| | First and second cuttings | 7 | −.3486 |
| | Third cutting | 7 | −.1787 |
| | First, second, and third cuttings | 7 | −.3249 |
| | | | *1913–1914* |
| 1922 | One cutting | 9 | +0.1630 |
| 1923 | First cutting | 9 | −.1739 |
| | Third cutting | 9 | −.2839 |
| | First and third cuttings | 9 | −.2477 |
| 1924 | Second cutting | 9 | −.2978 |
| | Third cutting | 9 | −.4413 |
| | Second and third cuttings | 9 | −.4071 |

The average values of these correlations of yields of alfalfa with other yields of alfalfa are set forth in Table 9.

With the exception of the correlations based on the single cutting for 1912 and 1922, these averages are negative throughout.

It is clear, therefore, that plots which showed superiority in alfalfa-yielding capacity in 1913 and 1914 proved on an average inferior in 1923 and 1924.

The final explanation of these results, in the opinion of the writers, must await the analysis of further data of agronomic experimentation. It is proper, however, to suggest possible explanations of observed results, since such tentative interpretations may serve to guide further work.

First of all, it is conceivable that wholly accidental differences of stand in these two periods (1912–1914 and 1922–1924) might be a source of such correlations as those which have been demonstrated. Other explanations, however, seem more probable.

The space and elevation relations of the field have already been discussed. It has been shown that the field is located within a loop of the main canal of a project that carries 400 second-feet of water. The water in this canal is 5 to 6 feet above the level of the field. The land slopes to the north and west. The southeast corner of the field, the high corner, is within 100 feet of the main canal. A shallow open ditch touches the southwest corner of the field, but curves away to the northwest.

There is a layer of sandy subsoil of possibly an acre in extent but of undetermined thickness which underlies these experimental areas. There is now definite evidence of seepage from the main canal through this sandy subsoil. A number of observation wells have been sunk around the field, three near the southeast corner and five along the north border, by which the rise and fall of the underground water may be observed. These observations have been made since the spring of 1923 only. Before that time no information was avail-

able as to the fluctuations of water in the subsoil, which is probably a phenomenon of relatively recent occurrence due to erosion in the main canal. There is reason to believe that the underground water has been rising each summer in recent years higher than it did in the earlier years of the experiment. The annual fluctuation of this underground water table is approximately 6 to 9 feet. It reaches its high point in August or September and its low point in April. In recent years the subsoil has been saturated at 1 or 2 feet below the surface in the southeast corner of the field, and it is believed that the root zone has been materially restricted throughout most of the east series (Series II).

The relationship between the yields of different portions of the field may be brought out roughly by computing the relative yields of different areas of the field. Retention of the yields of the individual plots (46 in number) results in such confusing irregularities that it is necessary to reduce the number of the plots in order to eliminate in some measure these individual variations. Since there are 23 plots in each of the two series, grouping into larger plots can be made only by taking fractions of the yields of certain of the plots occupying intermediate positions. Each of the two series has been divided into four larger plots by grouping as follows:

(1) Plots 1 to 5 plus 75 per cent of plot 6.
(2) Plots 7 to 11 plus 25 per cent of plot 6 plus 50 per cent of plot 12.
(3) Plots 13 to 17 plus 50 per cent of plot 12 plus 25 per cent of plot 18.
(4) Plots 18 to 23 plus 75 per cent of plot 18.

This divides the whole field into eight larger plots of uniform size. After obtaining the total yields for areas of quarters of each of the two sections (eighths of the entire field), the yields have been expressed as percentages of the average yields of the eight plots. Such percentages show at a glance whether the yield of the individual plots is lower (percentages less than 100) or higher (percentages over 100) than those of the field as a whole. The numerical values can best be expressed graphically as in Figures 5 and 6. In these diagrams the four groupings of plots on the two sections of the field are represented by four panels. The relative yields for Series II are represented by solid dots connected by solid lines. The relative yields of Series III are represented by circles connected by broken lines. These yields are distributed above and below the heavy transverse bars representing 100 per cent of the average yields for the areas as a whole. Dots or circles falling below the heavy line, therefore, represent lower yields than those which are normal for the field as a whole for the given crop and year. Circles or dots falling above the line indicate yields which are above the normal for the given crop. Inspection of these diagrams shows at once that the results for sugar beets in the eight larger plots are irregular. This is in accordance with other findings for sugar beets, and the point need not be considered further.

Turning to alfalfa, which has been shown to be of particular interest, it is noted that for the first group of alfalfa yields (1912–1914) the yields per plot are in general higher than the average for the field as a whole in the case of Series II. In the case of the stand for 1922 to 1924 the reverse appears to be true in three of the four comparisons of the results for Series II and III.

This result is a somewhat different expression for the negative correlation between the alfalfa yields of the first and second series as indicated above. It extends these results by indicating that whereas in the earlier crops Series II was better for alfalfa, Series III was better for alfalfa in the later period. The writers feel inclined

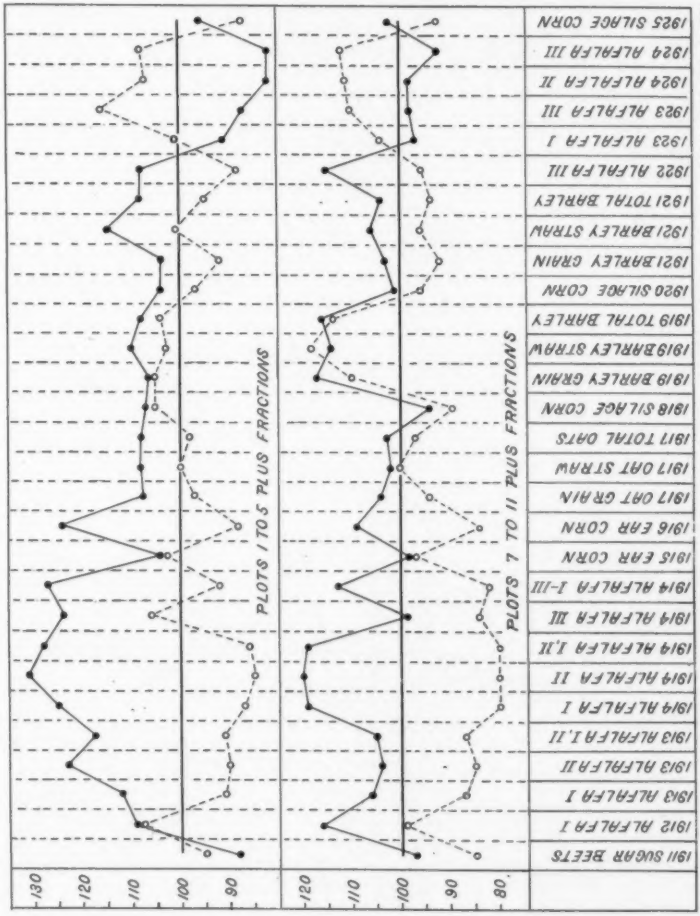


FIG. 5.—Distributions of relative (percentage) yields in 8 larger plots resulting from the combination of the 23 plots of Series II and III of Field B into 4 equal plots for each series. Solid dots and firm lines represent the relative yields of the 4 plots of Series II. Circles and broken lines represent the relative yields of the 4 plots of Series III. This figure represents the yields for the north half of the two series. (Compare fig. 6)

to suggest that in the earlier experiments the height of the water table had no harmful effect upon a deep-rooted crop such as alfalfa. It is quite possible that during drier periods the higher water table actually favored alfalfa growth on Series II. The higher water tables of recent years have probably had a deleterious influence,

which has been especially marked on Series II, where the water apparently comes nearer to the surface than in Series III.

Turning to relative yields of other crops for the period 1915 to 1921, it is noted that they are clustered irregularly but more closely around the 100 per cent line indicating normal yield. This suggests

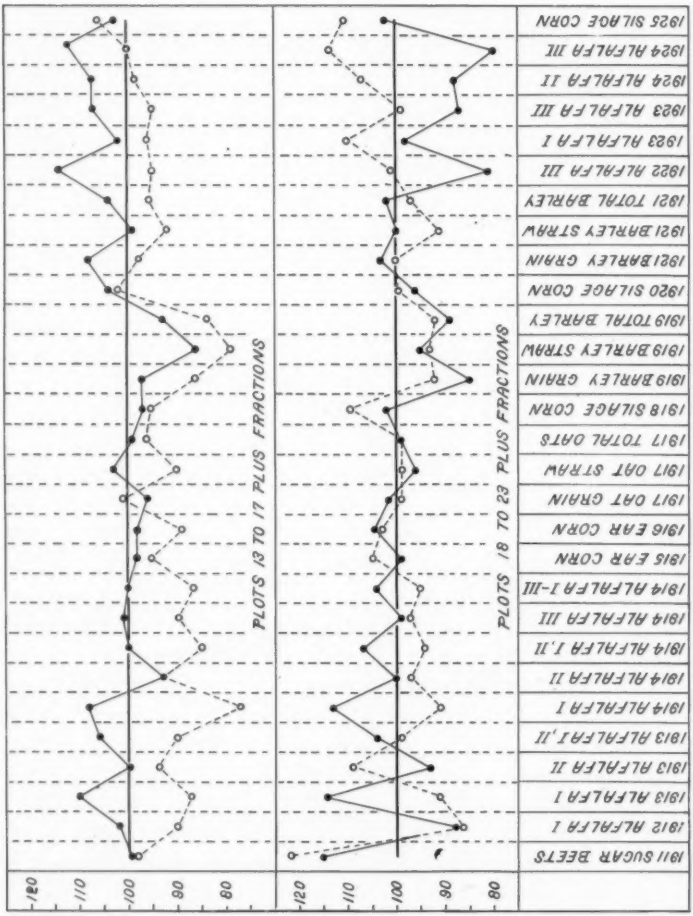


FIG. 6.—Distributions of relative (percentage) yields in 8 larger plots resulting from the combination of the 23 plots of Series II and III of field B into 4 equal plots for each series. Solid dots and firm lines represent the relative yields of the 4 plots of Series II. Circles and broken lines represent the relative yields of the 4 plots of Series III. This figure represents the yields for the south half of the two series. (Compare fig. 5)

that these more shallow-rooted crops have not been influenced in the same way as the deeper rooted alfalfa crops by the seepage water. The first crop of alfalfa in the case of both of the two stands gives results more in accordance with those for the annual crops than do the cuttings for the second and third years. Possibly this may be due to a more superficial root system in the first year.

## SUMMARY

This paper gives the results of a biometric analysis of crop yields obtained over a period of 15 years in a uniform cropping experiment conducted by the Office of Western Irrigation Agriculture at Huntley, Mont., during the years 1911 to 1925. The records are considered with particular reference to crops grown during the period 1920 to 1925.

The results show clearly that the problem of the permanence of the heterogeneity of the agricultural experimental field is capable of treatment by the method of interannual correlation.

In general it appears that, even in small experimental tracts, there is a positive correlation between the yields of a series of plots throughout a period of years. In other words, plots which show a heavier yield one year will in general show heavier yields in other years during the period under investigation.

Under some conditions, however, negative correlations may be found—i. e., plots which produce superior yields under the conditions of one year may produce on an average inferior yields another year. In the case of the present experiments it has been suggested that fluctuations in the level of the water table in irrigated land may play a large part in determining the relative crop-yielding capacity of the different plots. A deep-rooted crop, in the present case alfalfa, seems to be more affected by these conditions than the cereals.

The importance of these results for agricultural experimentation is increased rather than diminished by the finding of significant negative as well as positive relationships between the yields of crops in different years. Both positive and negative correlations may indicate the importance of a preceding crop in determining the characteristics of an experimental field. Studies in this field have been made by Hartwell and Damon (*8*, *9*), by Hartwell, Pember, and Merkle (*10*), by Garner, Lunn, and Brown (*1*), and by others. On the other hand, such correlations may indicate changing environmental conditions such as may be induced by variations in the level of the water table in the case of irrigation agriculture. Too great caution can not be exercised by agronomists in the selection of plots for tillage, fertilizer, or variety tests. It is clear from these results that not merely the physical and chemical characteristics of the soil but the nature of the preceding crop and changes in physical conditions from year to year must be taken into account in planning any such experiments.

Finally, it will be at once evident to agriculturists that the methods here employed may be of great value in the investigation of the after effect of the growth on land of a particular crop, or of temporarily changed physical conditions.

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# USE OF THE REFRACTOMETER IN THE ANALYSIS OF INDIVIDUAL SUGAR BEETS[1]

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## INTRODUCTION

Breeding and valuing sugar beets, especially those to be planted for the production of seed, entails the analysis of very large numbers of individuals. Criteria ordinarily used for judging the quality of commercial beets intended for the manufacture of sugar include the percentage of sucrose in the beet, the percentage of sucrose in the juice, the Brix (percentage of total soluble solids by the Brix hydrometer) of the juice, and the apparent purity (percentage of sucrose by direct polarization÷Brix) of the juice, but the only analytical criterion heretofore used for valuing seed beets appears to have been the percentage of sucrose in the beet. It is believed that the small size of the sample available has been the reason for limiting the analysis to this single determination. In order to minimize the mutilation of the beet and shock to it, the size of the sample removed must be restricted to the minimum quantity that will suffice for the determination of sucrose, and after the normal weight for the determination has been removed the residual sample is so small—frequently not more than 2 or 3 gm.—that the quantity of juice that may be obtained from it does not suffice for the determination of Brix or of specific gravity. The two methods available for sampling individual beets, one involving boring a hole through the beet and the other the removal of a wedge-shaped section extending from crown to tail, have been in use for many years, and various adaptations of these methods have been described by Beaudet (*1*),[2] Clark (*3*), Pellet (*7*), Pack (*6*), Saillard (*10*), Sherwood (*11*), and others. In all cases the sample is obtained as pulp in an exceedingly fine state of division.

At the suggestion of Dean A. Pack, associate agronomist at the Salt Lake City (Utah) field station of the Office of Sugar Plants, the writer investigated the adaptability of the refractometer for the determination of total soluble solids and apparent purity in juice from small samples removed from individual beets.

The writer has been able to find only two references relative to the subject. Komers (*4*) determined the percentage of sucrose in a series of samples of beet juice by means of the polariscope and the

[1] Received for publication Sept. 29, 1927; issued February, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 51.

percentage of total soluble solids by means of the refractometer. He subtracted the percentage of sucrose from the percentage of solids and considered the result as the percentage of nonsugar. The average of the nonsugar percentages was then used as a constant factor and subtracted from the percentage of solids by refractometer in individual samples as affording the percentage of sucrose. In a few cases the results agreed exactly with true sucrose as determined by the polariscope, but in most cases there was a plus or a minus variation, the plus variations ranging from 0.2 to 1.8 per cent and the minus ones from 0.1 to 0.9 per cent. He concluded that the method might be used for very rough approximation of sucrose and that it was superior to the ancient method of determining the comparative specific gravity of beets by floating them in salt solutions of definite specific gravity. He states that for careful and exact work the sucrose must be determined by means of the polariscope. Munerati and Mezzadroli (*5*) practically repeated the investigations of Komers and arrived at the same conclusions. Neither of these investigators mentions having attempted to adapt the solids by refractometer to the calculation of apparent purity, or having compared the percentage of solids by refractometer with the percentage of solids by the Brix hydrometer.

## SOURCES OF BEETS USED IN EXPERIMENTAL WORK

The beets used in the experimental work here reported were from the following sources:

Colorado beets: From northern Colorado, crop of 1923. Harvested in October, 1923, packed in damp earth, and stored in earthen storage cellar at just above freezing temperature until such time as refractometer investigations could be carried on (April, 1924). No doubt the long period of storage was responsible for the low percentages of sucrose. Otherwise the beets were in excellent condition.

Utah beets: From Salt Lake Valley, crop of 1924. Freshly harvested.

California beets: From the extreme southern beet-growing section, crop of 1924. Freshly harvested.

Virginia beets: From the Arlington Experiment Farm, Rosslyn, Va., crop of 1925 (experimental plots). Freshly harvested.

## USE OF THE REFRACTOMETER

The initial investigation included the study of the effect upon the refractive index and the corresponding total soluble solids of juice expressed at different pressures and of juice expressed after different lengths of time up to 24 hours. The pulp used was in an exceedingly fine state of division, corresponding to that produced by either of the types of rasps used for sampling. After thorough mixing, 5 to 8 gm. were placed in a small piece of linen cloth, and the juice was expressed by squeezing with the fingers. The first two or three drops of juice were permitted to fall directly upon the prism of the refractometer, and the index was read. All of the juice that it was possible to express by energetic squeezing and twisting was then collected in a vessel, mixed, and the index read. The last two or three drops that it was possible to express by extreme pressing and twisting were permitted to fall directly upon the prism, and the index was read.

Portions of the samples of pulp were placed in cans provided with tight-fitting covers and stored in a refrigerator at approximately 12° C. At the expiration of the time noted in the tables the above-described procedure was repeated.

An Abbe heatable prism refractometer was used. The light source consisted of a 50-watt mazda lamp in a box 6 by 6 by 6 inches lined with white asbestos paper and provided with a wide slot permitting light to reach the mirror of the instrument. A pane of glass was interposed between the box and the instrument in order to avoid the effect of heat from the lamp. The accuracy of the Brix hydrometers had been certified by the Bureau of Standards, and all thermometers used had been checked against thermometers the accuracy of which had been certified by the same bureau. The percentage of solids was obtained by the use of the table of Main (*2*) for water and solids and the correction table of Stanek (*2*) for temperature.

TABLE 1.—*Refractive index and total solids in juice by refractometer from stored Colorado beets*

| Description of sample | Juice from fresh pulp | | Juice from pulp 2 hours old | | Juice from pulp 24 hours old | | Percentage of total solids at 20° C. in juice from— | | |
|---|---|---|---|---|---|---|---|---|---|
| | Index | Temperature, °C. | Index | Temperature, °C. | Index | Temperature, °C. | Fresh pulp | Pulp 2 hours old | Pulp 24 hours old |
| Light pressure: | | | | | | | | | |
| No. 1 | 1.3541 | 24 | 1.3542 | 24.5 | 1.3539 | 25.5 | 14.46 | 14.54 | 14.40 |
| No. 2 | 1.3547 | 24 | 1.3545 | 24.5 | 1.3540 | 26 | 14.69 | 14.69 | 14.49 |
| No. 3 | 1.3535 | 24.5 | 1.3537 | 24.5 | 1.3533 | 26 | 14.09 | 14.19 | 14.09 |
| No. 4 | 1.3525 | 24.5 | 1.3525 | 24.5 | 1.3522 | 26 | 13.44 | 13.44 | 13.39 |
| No. 5 | 1.3560 | 24.5 | 1.3560 | 24.5 | 1.3552 | 26 | 15.64 | 15.64 | 15.29 |
| No. 6 | 1.3568 | 24.5 | 1.3568 | 24.5 | 1.3561 | 26 | 16.14 | 16.14 | 15.79 |
| No. 7 | 1.3573 | 24.5 | 1.3575 | 24.5 | 1.3570 | 26 | 16.44 | 16.54 | 16.34 |
| No. 8 | 1.3563 | 24.5 | 1.3563 | 24.5 | 1.3558 | 26.5 | 15.84 | 15.84 | 15.68 |
| No. 9 | 1.3539 | 24.5 | 1.3539 | 24.5 | 1.3530 | 26.5 | 14.34 | 14.34 | 13.93 |
| Average | 1.3550 | | 1.3550 | | 1.3545 | | 15.02 | 15.04 | 14.82 |
| No. 10 | 1.3575 | 25 | | | | | 16.57 | | |
| No. 11 | 1.3558 | 25 | | | | | 15.57 | | |
| No. 12 | 1.3568 | 25 | | | | | 16.17 | | |
| No. 13 | 1.3555 | 25 | | | | | 15.37 | | |
| No. 14 | 1.3537 | 25 | | | | | 14.22 | | |
| No. 15 | 1.3555 | 25 | | | | | 15.37 | | |
| No. 16 | 1.3528 | 25 | | | | | 13.67 | | |
| No. 17 | 1.3515 | 25 | | | | | 12.82 | | |
| No. 18 | 1.3538 | 25 | | | | | 14.32 | | |
| No. 19 | 1.3543 | 25 | | | | | 14.62 | | |
| Average | 1.3547 | | | | | | 14.87 | | |
| No. 20 | 1.3548 | 25 | | | 1.3546 | 25 | 14.92 | | 14.82 |
| No. 21 | 1.3541 | 25 | | | 1.3540 | 25 | 14.52 | | 14.42 |
| No. 22 | 1.3548 | 25 | | | 1.3545 | 25 | 14.92 | | 14.72 |
| No. 23 | 1.3530 | 25 | | | 1.3530 | 25 | 13.82 | | 13.82 |
| No. 24 | 1.3531 | 25 | | | 1.3531 | 25 | 13.87 | | 13.87 |
| No. 25 | 1.3560 | 25 | | | 1.3562 | 25 | 15.67 | | 15.82 |
| No. 26 | 1.3540 | 25 | | | 1.3543 | 25 | 14.42 | | 14.62 |
| No. 27 | 1.3555 | 25 | | | 1.3552 | 25 | 15.37 | | 15.22 |
| No. 28 | 1.3552 | 25 | | | 1.3558 | 25 | 15.22 | | 15.57 |
| No. 29 | 1.3544 | 25 | | | 1.3550 | 25 | 14.67 | | 15.07 |
| Average | 1.3545 | | | | 1.3546 | | 14.74 | | 14.80 |

TABLE 1.—*Refractive index and total solids in juice by refractometer from stored Colorado beets*—Continued

| Description of sample | Juice from fresh pulp | | Juice from pulp 2 hours old | | Juice from pulp 24 hours old | | Percentage of total solids at 20° C. in juice from— | | |
|---|---|---|---|---|---|---|---|---|---|
| | Index | Temperature, °C. | Index | Temperature, °C. | Index | Temperature, °C. | Fresh pulp | Pulp 2 hours old | Pulp 24 hours old |
| Heavy pressure: | | | | | | | | | |
| No. 1 | 1.3535 | 24 | 1.3535 | 24.5 | 1.3533 | 25.5 | 14.06 | 14.09 | 14.05 |
| No. 2 | 1.3539 | 24 | 1.3539 | 24.5 | 1.3534 | 26 | 14.31 | 14.34 | 14.14 |
| No. 3 | 1.3530 | 24.5 | 1.3530 | 24.5 | 1.3526 | 26 | 13.79 | 13.79 | 13.59 |
| No. 4 | 1.3521 | 24.5 | 1.3520 | 24.5 | 1.3519 | 26 | 13.19 | 13.14 | 13.19 |
| No. 5 | 1.3550 | 24.5 | 1.3550 | 24.5 | 1.3548 | 26 | 15.04 | 15.04 | 14.99 |
| No. 6 | 1.3563 | 24.5 | 1.3562 | 24.5 | 1.3555 | 26 | 15.84 | 15.79 | 15.44 |
| No. 7 | 1.3570 | 24.5 | 1.3570 | 24.5 | 1.3565 | 26 | 16.24 | 16.24 | 16.04 |
| No. 8 | 1.3559 | 24.5 | 1.3559 | 24.5 | 1.3555 | 26.5 | 15.59 | 15.59 | 15.47 |
| No. 9 | 1.3530 | 24.5 | 1.3530 | 24.5 | 1.3523 | 26.5 | 13.79 | 13.79 | 13.47 |
| Average | 1.3544 | | 1.3544 | | 1.3540 | | 14.65 | 14.65 | 14.49 |
| No. 10 | 1.3571 | 25 | | | | | 16.32 | | |
| No. 11 | 1.3555 | 25 | | | | | 15.37 | | |
| No. 12 | 1.3568 | 25 | | | | | 16.17 | | |
| No. 13 | 1.3553 | 25 | | | | | 15.27 | | |
| No. 14 | 1.3530 | 25 | | | | | 13.82 | | |
| No. 15 | 1.3553 | 25 | | | | | 15.27 | | |
| No. 16 | 1.3525 | 25 | | | | | 13.47 | | |
| No. 17 | 1.3511 | 25 | | | | | 12.62 | | |
| No. 18 | 1.3535 | 25 | | | | | 14.12 | | |
| No. 19 | 1.3540 | 25 | | | | | 14.42 | | |
| Average | 1.3544 | | | | | | 14.68 | | |
| No. 20 | 1.3543 | 25 | | | 1.3542 | 25 | 14.62 | | 14.57 |
| No. 21 | 1.3541 | 25 | | | 1.3539 | 25 | 14.52 | | 14.37 |
| No. 22 | 1.3548 | 25 | | | 1.3549 | 25 | 14.92 | | 14.72 |
| No. 23 | 1.3530 | 25 | | | 1.3520 | 25 | 13.82 | | 13.17 |
| No. 24 | 1.3531 | 25 | | | 1.3529 | 25 | 13.87 | | 13.72 |
| No. 25 | 1.3562 | 25 | | | 1.3562 | 25 | 15.82 | | 15.82 |
| No. 26 | 1.3539 | 25 | | | 1.3540 | 25 | 14.37 | | 14.42 |
| No. 27 | 1.3550 | 25 | | | 1.3548 | 25 | 15.07 | | 14.92 |
| No. 28 | 1.3552 | 25 | | | 1.3555 | 25 | 15.22 | | 15.37 |
| No. 29 | 1.3544 | 25 | | | 1.3544 | 25 | 14.67 | | 14.67 |
| Average | 1.3544 | | | | 1.3542 | | 14.69 | | 14.57 |
| Total mixed juice: | | | | | | | | | |
| No. 1 | 1.3540 | 24 | 1.3540 | 24.5 | 1.3538 | 25.5 | 14.36 | 14.39 | 14.35 |
| No. 2 | 1.3543 | 24 | 1.3543 | 24.5 | 1.3540 | 26 | 14.56 | 14.59 | 14.49 |
| No. 3 | 1.3535 | 24.5 | 1.3535 | 24.5 | 1.3531 | 26 | 14.09 | 14.09 | 13.94 |
| No. 4 | 1.3525 | 24.5 | 1.3525 | 24.5 | 1.3522 | 26 | 13.44 | 13.44 | 13.39 |
| No. 5 | 1.3560 | 24.5 | 1.3560 | 24.5 | 1.3551 | 26 | 15.64 | 15.64 | 15.19 |
| No. 6 | 1.3568 | 24.5 | 1.3568 | 24.5 | 1.3561 | 26 | 16.14 | 16.14 | 15.79 |
| No. 7 | 1.3573 | 24.5 | 1.3575 | 24.5 | 1.3570 | 26 | 16.44 | 16.54 | 16.34 |
| No. 8 | 1.3563 | 24.5 | 1.3563 | 24.5 | 1.3558 | 26.5 | 15.84 | 15.84 | 15.67 |
| No. 9 | 1.3539 | 24.5 | 1.3539 | 24.5 | 1.3530 | 26.5 | 14.34 | 14.34 | 13.92 |
| Average | 1.3550 | | 1.3550 | | 1.3545 | | 14.98 | 15.00 | 14.79 |
| No. 10 | 1.3575 | 25 | | | | | 16.57 | | |
| No. 11 | 1.3558 | 25 | | | | | 15.57 | | |
| No. 12 | 1.3568 | 25 | | | | | 16.17 | | |
| No. 13 | 1.3555 | 25 | | | | | 15.37 | | |
| No. 14 | 1.3537 | 25 | | | | | 14.22 | | |
| No. 15 | 1.3555 | 25 | | | | | 15.37 | | |
| No. 16 | 1.3528 | 25 | | | | | 13.67 | | |
| No. 17 | 1.3515 | 25 | | | | | 12.82 | | |
| No. 18 | 1.3538 | 25 | | | | | 14.32 | | |
| No. 19 | 1.3543 | 25 | | | | | 14.62 | | |
| Average | 1.3547 | | | | | | 14.87 | | |

TABLE 2.—*Refractive index and total solids in juice from fresh pulp from crown, middle, and tail sections of freshly harvested California beets*

[Each section was pulped separately and juice expressed as indicated. Determinations were made at 20° C.]

| Description of sample | Light pressure | | Heavy pressure | | Total mixed juice | |
|---|---|---|---|---|---|---|
| | Index | Percentage of solids | Index | Percentage of solids | Index | Percentage of solids |
| No. 1 Crown | 1.3623 | 19.15 | 1.3611 | 18.45 | 1.3620 | 19.00 |
| No. 1 Middle | 1.3610 | 18.40 18.08 | 1.3600 | 17.80 17.50 | 1.3610 | 18.40 17.98 |
| No. 1 Tail | 1.3582 | 16.70 | 1.3575 | 16.25 | 1.3580 | 16.55 |
| No. 2 Crown | 1.3630 | 19.60 | 1.3625 | 19.30 | 1.3629 | 19.50 |
| No. 2 Middle | 1.3635 | 19.90 19.90 | 1.3629 | 19.50 19.62 | 1.3633 | 19.75 19.82 |
| No. 2 Tail | 1.3640 | 20.20 | 1.3628 | 20.05 | 1.3640 | 20.20 |
| No. 3 Crown | 1.3665 | 21.65 | 1.3660 | 21.35 | 1.3665 | 21.65 |
| No. 3 Middle | 1.3660 | 21.35 21.45 | 1.3657 | 21.20 21.12 | 1.3660 | 21.35 21.42 |
| No. 3 Tail | 1.3660 | 21.35 | 1.3650 | 20.80 | 1.3658 | 21.25 |
| No. 4 Crown | 1.3615 | 18.70 | 1.3610 | 18.40 | 1.3615 | 18.70 |
| No. 4 Middle | 1.3613 | 18.55 18.13 | 1.3609 | 18.30 17.95 | 1.3612 | 18.50 18.17 |
| No. 4 Tail | 1.3590 | 17.15 | 1.3590 | 17.15 | 1.3592 | 17.30 |
| No. 5 Crown | 1.3618 | 18.85 | 1.3610 | 18.40 | 1.3618 | 18.85 |
| No. 5 Middle | 1.3620 | 19.00 19.05 | 1.3616 | 18.75 18.67 | 1.3620 | 19.00 19.05 |
| No. 5 Tail | 1.3625 | 19.30 | 1.3618 | 18.85 | 1.3625 | 19.30 |
| Average | 1.36257 | 19.32 | 1.36192 | 18.97 | 1.36251 | 19.29 |
| Average Crown | | 19.59 | | 19.18 | | 19.54 |
| Average Middle | | 19.44 | | 19.11 | | 19.40 |
| Average Tail | | 18.94 | | 18.62 | | 18.92 |

Referring to the results in Tables 1 and 2, it will be noted that no difference exists between the refractive index and the total soluble solids in juice from light pressure (initial juice) and in total mixed juice, and that slightly lower results are obtained in the case of juice expressed by heavy pressure (residual juice). It is considered that the lower results in this juice were due to the effect of the so-called "colloidal water" ("colloidwasser"), which has been fully described by Rümpler (*9*). The quantity of this juice is so small and the difference in results so slight that its admixture with the comparatively very great quantity of total mixed juice could not result in a detectable change in the refractive index. It will also be noted that no appreciable difference exists between the results on fresh pulp and on pulp that has stood for 2 hours and for 24 hours, the very slight differences that do occur being within the operative error. In a recent communication Dean A. Pack has stated that he was able to find no difference between determinations separated by an interval of 24 to 48 hours.

It does not appear to be necessary to exert heavy pressure or to express the juice through cloth, and it is concluded that results obtained by squeezing pulp with the fingers and permitting a few drops of juice to fall directly upon the prism of the refractometer—no difficulty is encountered in obtaining a reading on this juice—afford a correct representation of the total soluble solids by refractometer in the normal juice in the pulp. It is also concluded that when the pulp is stored in a refrigerator in air-tight containers the determinations may be delayed with safety up to a period of 24 hours.

## COMPARISON OF TOTAL SOLIDS BY REFRACTOMETER AND BY BRIX HYDROMETER

One or more entire beets were pulped and the juice expressed by means of a small hand-operated screw press. After removal of occluded air and very thorough mixing the Brix reading was obtained, followed by immediate determination of the refractive index and of the percentage of sucrose by direct polarization. The results are given in Table 3.

TABLE 3.—*Total solids and purity in juice by Brix hydrometer and by refractometer from beets from different sources*

[The percentage of sucrose given in column 2 was determined by direct polarization. In computing the approximate percentage of sucrose shown in column 11 the solids by refractometer are multiplied by the average purity by refractometer. These multipliers are 84.9 and 82 per cent (column 7) for Utah beets and for California beets, respectively. The Colorado beets were from storage; all others were freshly harvested]

| Source and sample | Percentage of sucrose | Percentage of solids at 17.5° C. | | | Purity | | | Approximate nonsugars | | Approximate percentage of sucrose | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brix | Refractometer | Variation, refractometer from Brix | Brix | Refractometer | Variation, refractometer from Brix | Brix—sucrose | Solids by refractometer—sucrose | As computed | Variation from real sucrose |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Colorado beets: | | | | | | | | | | | |
| No. 1 | 8.45 | 15.70 | 15.35 | −0.35 | 53.8 | 55.0 | +1.2 | 7.25 | 6.90 | ------ | ------ |
| No. 2 | 10.20 | 14.65 | 14.20 | −.45 | 69.6 | 71.8 | +2.2 | 4.45 | 4.00 | ------ | ------ |
| No. 3 | 8.60 | 13.20 | 12.60 | −.60 | 65.2 | 68.3 | +3.1 | 4.60 | 4.00 | ------ | ------ |
| No. 4 | 7.25 | 12.55 | 12.30 | −.25 | 57.8 | 58.9 | +1.1 | 5.30 | 5.05 | ------ | ------ |
| No. 5 | 10.75 | 14.45 | 14.30 | −.15 | 74.4 | 75.2 | +.8 | 3.70 | 3.55 | ------ | ------ |
| No. 6 | 12.40 | 15.90 | 15.65 | −.25 | 78.0 | 79.2 | +1.2 | 3.50 | 3.25 | ------ | ------ |
| No. 7 | 10.30 | 14.35 | 14.10 | −.25 | 71.8 | 73.0 | +1.2 | 4.05 | 3.80 | ------ | ------ |
| No. 8 | 10.00 | 14.15 | 13.90 | −.25 | 70.7 | 71.9 | +1.2 | 4.15 | 3.90 | ------ | ------ |
| No. 9 | 7.10 | 11.95 | 11.19 | −.76 | 59.4 | 63.4 | +4.0 | 4.85 | 4.09 | ------ | ------ |
| No. 10 | 10.45 | 13.80 | 13.31 | −.49 | 75.7 | 78.5 | +2.8 | 3.35 | 2.86 | ------ | ------ |
| No. 11 | 11.40 | 14.45 | 14.16 | −.29 | 78.9 | 80.5 | +1.6 | 3.05 | 2.76 | ------ | ------ |
| No. 12 | 6.30 | 12.25 | 12.04 | −.21 | 51.4 | 52.3 | +.9 | 5.95 | 5.74 | ------ | ------ |
| No. 13 | 9.40 | 13.45 | 12.94 | −.51 | 69.9 | 72.6 | +2.7 | 4.05 | 3.54 | ------ | ------ |
| No. 14 | 10.65 | 14.70 | 14.46 | −.24 | 72.4 | 73.7 | +1.3 | 4.05 | 3.81 | ------ | ------ |
| No. 15 | 12.90 | 15.95 | 15.81 | −.14 | 80.9 | 81.6 | +.7 | 3.05 | 2.91 | ------ | ------ |
| No. 16 | 4.20 | 11.73 | 11.42 | −.31 | 35.8 | 36.8 | +1.0 | 7.53 | 7.22 | ------ | ------ |
| No. 17 | 7.40 | 12.83 | 12.69 | −.14 | 57.7 | 58.3 | +.6 | 5.43 | 5.29 | ------ | ------ |
| No. 18 | 7.65 | 13.45 | 13.34 | −.11 | 56.9 | 57.3 | +.4 | 5.80 | 5.69 | ------ | ------ |
| No. 19 | 10.70 | 15.30 | 15.24 | −.06 | 69.9 | 70.2 | +.3 | 4.60 | 4.54 | ------ | ------ |
| No. 20 | 11.50 | 15.80 | 15.69 | −.11 | 72.8 | 73.3 | +.5 | 4.30 | 4.19 | ------ | ------ |
| No. 21 | 11.80 | 15.75 | 15.54 | −.21 | 74.9 | 75.9 | +1.0 | 3.95 | 3.74 | ------ | ------ |
| No. 22 | 10.80 | 14.60 | 14.59 | −.01 | 74.0 | 74.0 | 0 | 3.80 | 3.79 | ------ | ------ |
| No. 23 | 10.60 | 14.15 | 13.99 | −.16 | 74.9 | 75.8 | +.9 | 3.55 | 3.39 | ------ | ------ |
| No. 24 | 11.30 | 14.85 | 14.59 | −.26 | 76.1 | 77.5 | +1.4 | 3.55 | 3.29 | ------ | ------ |
| No. 25 | 7.50 | 11.38 | 10.92 | −.46 | 65.9 | 68.7 | +2.8 | 3.88 | 3.42 | ------ | ------ |
| No. 26 | 8.40 | 11.93 | 11.42 | −.51 | 70.4 | 73.6 | +3.2 | 3.53 | 3.02 | ------ | ------ |
| No. 27 | 10.20 | 14.27 | 14.09 | −.18 | 71.5 | 72.4 | +.9 | 4.07 | 3.89 | ------ | ------ |
| No. 28 | 8.00 | 12.45 | 12.12 | −.33 | 64.3 | 66.0 | +1.7 | 4.45 | 4.12 | ------ | ------ |
| No. 29 | 8.75 | 12.76 | 12.47 | −.29 | 68.6 | 70.2 | +1.6 | 4.01 | 3.72 | ------ | ------ |
| No. 30 | 7.70 | 12.47 | 12.27 | −.20 | 61.7 | 62.8 | +1.1 | 4.77 | 4.57 | ------ | ------ |
| No. 31 | 7.40 | 12.32 | 12.07 | −.25 | 60.1 | 61.3 | +1.2 | 4.92 | 4.67 | ------ | ------ |
| No. 32 | 8.70 | 12.88 | 12.67 | −.21 | 67.5 | 68.7 | +1.2 | 4.18 | 3.97 | ------ | ------ |
| No. 33 | 6.70 | 13.17 | 12.94 | −.23 | 50.9 | 51.8 | +.9 | 6.47 | 6.24 | ------ | ------ |
| No. 34 | 9.20 | 13.27 | 13.09 | −.18 | 69.3 | 70.3 | +1.0 | 4.07 | 3.89 | ------ | ------ |
| No. 35 | 9.10 | 14.14 | 13.74 | −.40 | 64.4 | 66.2 | +1.8 | 5.04 | 4.64 | ------ | ------ |
| No. 36 | 10.10 | 13.99 | 13.74 | −.25 | 72.2 | 73.5 | +1.3 | 3.89 | 3.64 | ------ | ------ |
| No. 37 | 10.70 | 14.39 | 14.09 | −.30 | 74.4 | 75.9 | +1.5 | 3.69 | 3.39 | ------ | ------ |
| Average | 9.31 | 13.77 | 13.49 | −.28 | 67.1 | 68.6 | +1.4 | 4.46 | 4.18 | ------ | ------ |
| Maximum | 12.90 | 15.95 | 15.81 | −.76 | 80.9 | 81.6 | +4.0 | 7.53 | 7.22 | ------ | ------ |
| Minimum | 4.20 | 11.38 | 10.92 | −.01 | 35.8 | 36.8 | 0 | 3.05 | 2.76 | ------ | ------ |

TABLE 3.—*Total solids and purity in juice by Brix hydrometer and by refractometer from beets from different sources*—Continued

| Source and sample | Percentage of sucrose | Percentage of solids at 17.5° C. | | | Purity | | | Approximate nonsugars | | Approximate percentage of sucrose | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brix | Refractometer | Variation, refractometer from Brix | Brix | Refractometer | Variation, refractometer from Brix | Brix—sucrose | Solids by refractometer—sucrose | As computed | Variation from real sucrose |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Utah beets: | | | | | | | | | | | |
| No. 1 | 21.20 | 23.70 | 23.92 | +0.22 | 89.5 | 88.6 | −0.9 | 2.50 | 2.72 | 20.31 | −0.89 |
| No. 2 | 20.90 | 23.60 | 24.02 | +.42 | 88.6 | 87.0 | −1.6 | 2.70 | 3.12 | 20.39 | −.51 |
| No. 3 | 14.80 | 19.50 | 19.17 | −.33 | 75.9 | 77.2 | +1.3 | 4.70 | 4.37 | 16.28 | +1.48 |
| No. 4 | 14.75 | 19.50 | 19.17 | −.33 | 75.6 | 76.9 | +1.3 | 4.75 | 4.42 | 16.28 | +1.53 |
| No. 5 | 17.20 | 21.30 | 20.97 | −.33 | 80.8 | 82.0 | +1.2 | 4.10 | 3.77 | 17.80 | +.60 |
| No. 6 | 18.10 | 22.05 | 21.72 | −.33 | 82.1 | 83.3 | +1.2 | 3.95 | 3.62 | 18.44 | +.34 |
| No. 7 | 19.85 | 22.90 | 23.02 | +.12 | 86.7 | 86.2 | −.5 | 3.05 | 3.17 | 19.54 | −.31 |
| No. 8 | 20.90 | 23.70 | 23.92 | +.22 | 88.2 | 87.4 | −.8 | 2.80 | 3.02 | 20.31 | −.59 |
| No. 9 | 19.30 | 22.40 | 22.62 | +.22 | 86.2 | 85.3 | −.9 | 3.10 | 3.32 | 19.20 | −.10 |
| No. 10 | 19.20 | 22.30 | 22.72 | +.42 | 86.1 | 84.5 | −1.6 | 3.10 | 3.52 | 19.29 | +.09 |
| No. 11 | 19.40 | 22.60 | 22.87 | +.27 | 85.8 | 84.8 | −1.0 | 3.20 | 3.47 | 19.42 | +.02 |
| No. 12 | 19.40 | 22.20 | 22.27 | +.07 | 87.4 | 87.1 | −.3 | 2.80 | 2.87 | 18.91 | −.49 |
| No. 13 | 18.45 | 21.65 | 21.52 | −.13 | 85.2 | 85.7 | +.5 | 3.20 | 3.07 | 18.27 | −.18 |
| No. 14 | 20.80 | 23.55 | 23.92 | +.37 | 88.3 | 87.0 | −1.3 | 2.75 | 3.12 | 20.31 | −.49 |
| No. 15 | 20.60 | 22.75 | 22.92 | +.17 | 90.5 | 89.9 | −.6 | 2.15 | 2.32 | 19.46 | −1.14 |
| Average | 18.99 | 22.25 | 22.32 | +.07 | 85.1 | 84.9 | −.27 | 3.26 | 3.33 | 18.95 | −.04 |
| Maximum | 21.20 | 23.70 | 24.02 | +.42<br>−.33 | 90.5 | 89.9 | +1.3<br>−1.6 | 4.75 | 4.42 | ------ | +1.53<br>−1.14 |
| Minimum | 14.75 | 19.50 | 19.17 | +.07<br>−.13 | 75.6 | 76.9 | +.5<br>−.3 | 2.15 | 2.32 | ------ | +.02<br>−.10 |
| California beets: | | | | | | | | | | | |
| No. 1 | 13.50 | 16.55 | 16.77 | +.22 | 81.6 | 80.5 | −1.1 | 3.05 | 3.27 | 13.75 | +.25 |
| No. 2 | 14.35 | 17.15 | 17.37 | +.22 | 83.7 | 82.6 | −1.1 | 2.80 | 3.02 | 14.24 | −.11 |
| No. 3 | 14.70 | 17.85 | 18.17 | +.32 | 82.4 | 80.9 | −1.5 | 3.15 | 3.47 | 14.90 | +.20 |
| No. 4 | 14.80 | 17.75 | 17.92 | +.17 | 83.4 | 82.6 | −.8 | 2.95 | 3.12 | 14.69 | −.11 |
| No. 5 | 14.10 | 17.10 | 17.27 | +.17 | 82.5 | 81.6 | −.9 | 3.00 | 3.17 | 14.16 | +.06 |
| No. 6 | 16.00 | 18.85 | 18.82 | −.03 | 84.9 | 85.0 | +.1 | 2.85 | 2.82 | 15.43 | −.57 |
| No. 7 | 16.50 | 19.35 | 19.57 | +.22 | 85.3 | 84.3 | −1.0 | 2.85 | 3.07 | 16.05 | .45 |
| No. 8 | 15.70 | 18.95 | 18.87 | −.08 | 82.8 | 83.2 | +.4 | 3.25 | 3.17 | 15.47 | −.23 |
| No. 9 | 16.30 | 19.50 | 19.82 | +.32 | 83.6 | 82.2 | −1.4 | 3.20 | 3.52 | 16.25 | −.05 |
| No. 10 | 15.90 | 19.10 | 19.12 | +.02 | 83.2 | 83.2 | 0 | 3.20 | 3.22 | 15.68 | −.22 |
| No. 11 | 14.90 | 17.50 | 17.82 | +.32 | 85.1 | 83.6 | −1.5 | 2.60 | 2.92 | 14.61 | −.29 |
| No. 12 | 14.90 | 17.45 | 17.62 | +.17 | 85.4 | 84.6 | −.8 | 2.55 | 2.72 | 14.45 | −.45 |
| No. 13 | 15.90 | 18.45 | 18.57 | +.12 | 86.2 | 85.6 | −.6 | 2.55 | 2.67 | 15.23 | −.67 |
| No. 14 | 14.00 | 17.00 | 17.27 | +.27 | 82.4 | 81.1 | −1.3 | 3.00 | 3.27 | 14.16 | +.16 |
| No. 15 | 15.20 | 17.60 | 17.82 | +.22 | 86.4 | 85.3 | −1.1 | 2.40 | 2.62 | 14.61 | −.59 |
| No. 16 | 15.25 | 18.00 | 18.07 | +.07 | 84.7 | 84.4 | −.3 | 2.75 | 2.82 | 14.82 | −.43 |
| No. 17 | 15.35 | 18.10 | 18.07 | −.03 | 84.8 | 84.9 | +.1 | 2.75 | 2.72 | 14.82 | −.53 |
| No. 18 | 16.45 | 19.55 | 19.77 | +.22 | 84.1 | 83.2 | −.9 | 3.10 | 3.32 | 16.21 | −.24 |
| No. 19 | 13.30 | 16.50 | 16.97 | +.47 | 80.6 | 78.4 | −2.2 | 3.20 | 3.67 | 13.92 | +.62 |
| No. 20 | 13.45 | 16.55 | 16.72 | +.17 | 81.3 | 80.4 | −.9 | 3.10 | 3.27 | 13.71 | +.26 |
| No. 21 | 12.90 | 16.15 | 16.27 | +.12 | 79.9 | 79.3 | −.6 | 3.25 | 3.37 | 13.34 | +.44 |
| No. 22 | 12.65 | 17.45 | 17.62 | +.17 | 72.5 | 71.8 | −.7 | 4.80 | 4.97 | 14.45 | +1.80 |
| No. 23 | 13.60 | 17.55 | 17.62 | +.07 | 77.5 | 77.2 | −.3 | 3.95 | 4.02 | 14.45 | +.85 |
| Average | 14.77 | 17.83 | 18.00 | +.17 | 82.8 | 82.0 | −.8 | 3.06 | 3.23 | 14.76 | −.01 |
| Maximum | 16.50 | 19.55 | 19.82 | +.47<br>−.08 | 86.4 | 85.6 | +.4<br>−2.2 | 4.80 | 4.97 | ------ | +1.80<br>−.67 |
| Minimum | 12.65 | 16.15 | 16.27 | +.02<br>−.03 | 72.5 | 71.8 | +0<br>−0 | 2.40 | 2.62 | ------ | +.06<br>−.05 |

TABLE 3.—*Total solids and purity in juice by Brix hydrometer and by refractometer from beets from different sources*—Continued

| Source and sample | Percentage of sucrose | Percentage of solids at 17.5° C. | | | Purity | | | Approximate nonsugars | | Approximate percentage of sucrose | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brix | Refractometer | Variation, refractometer from Brix | Brix | Refractometer | Variation, refractometer from Brix | Brix−sucrose | Solids by refractometer−sucrose | As computed | Variation from real sucrose |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Virginia beets: | | | | | | | | | | | |
| No. 1 | 14.70 | 18.30 | 18.40 | +0.10 | 80.3 | 79.9 | −0.4 | 3.60 | 3.70 | | |
| No. 2 | 14.65 | 17.95 | 17.95 | 0 | 81.6 | 81.6 | 0 | 3.30 | 3.30 | | |
| No. 3 | 14.90 | 18.85 | 18.60 | −.25 | 79.0 | 80.1 | +1.1 | 3.95 | 3.70 | | |
| No. 4 | 14.60 | 17.90 | 18.10 | +.20 | 81.6 | 80.7 | −.9 | 3.30 | 3.50 | | |
| No. 5 | 15.55 | 19.10 | 19.14 | +.04 | 81.4 | 81.2 | −.2 | 3.55 | 3.59 | | |
| No. 6 | 14.40 | 17.90 | 17.74 | −.16 | 80.4 | 81.2 | +.8 | 3.50 | 3.34 | | |
| No. 7 | 14.80 | 18.20 | 18.30 | +.10 | 81.3 | 80.9 | −.4 | 3.40 | 3.50 | | |
| No. 8 | 15.70 | 18.85 | 19.06 | +.21 | 83.3 | 82.4 | −.9 | 3.15 | 3.36 | | |
| No. 9 | 16.00 | 19.35 | 19.62 | +.27 | 82.7 | 81.5 | −1.2 | 3.35 | 3.62 | | |
| No. 10 | 14.65 | 17.85 | 18.27 | +.42 | 82.1 | 80.2 | −1.9 | 3.20 | 3.62 | | |
| No. 11 | 15.50 | 18.80 | 18.90 | +.10 | 82.4 | 82.0 | −.4 | 3.30 | 3.40 | | |
| No. 12 | 14.85 | 18.65 | 18.75 | +.10 | 79.6 | 79.2 | −.4 | 3.80 | 3.90 | | |
| No. 13 | 14.70 | 18.60 | 18.60 | 0 | 79.0 | 79.0 | 0 | 3.90 | 3.90 | | |
| No. 14 | 14.85 | 18.70 | 18.84 | +.14 | 79.4 | 78.8 | −.6 | 3.85 | 3.99 | | |
| No. 15 | 13.70 | 17.30 | 17.18 | −.12 | 79.2 | 79.7 | +.5 | 3.60 | 3.48 | | |
| No. 16 | 14.15 | 17.65 | 17.73 | +.08 | 80.2 | 79.8 | −.4 | 3.50 | 3.58 | | |
| No. 17 | 14.00 | 17.65 | 17.58 | −.07 | 79.3 | 79.6 | +.3 | 3.65 | 3.58 | | |
| No. 18 | 14.35 | 18.35 | 18.40 | +.05 | 78.2 | 78.0 | −.2 | 4.00 | 4.05 | | |
| No. 19 | 14.50 | 17.95 | 17.69 | −.26 | 80.8 | 82.0 | +1.2 | 3.45 | 3.19 | | |
| No. 20 | 15.20 | 18.25 | 18.45 | +.20 | 83.3 | 82.4 | −.9 | 3.05 | 3.25 | | |
| No. 21 | 14.25 | 17.80 | 18.12 | +.32 | 80.1 | 78.6 | −1.5 | 3.55 | 3.87 | | |
| No. 22 | 14.55 | 18.00 | 18.30 | +.30 | 80.8 | 79.5 | −1.3 | 3.45 | 3.75 | | |
| No. 23 | 14.60 | 18.20 | 18.15 | −.05 | 80.2 | 80.4 | +.2 | 3.60 | 3.55 | | |
| No. 24 | 15.25 | 18.75 | 19.00 | +.25 | 81.3 | 80.3 | −1.0 | 3.50 | 3.75 | | |
| No. 25 | 14.20 | 17.65 | 17.80 | +.15 | 80.5 | 79.8 | −.7 | 3.45 | 3.60 | | |
| No. 26 | 14.10 | 17.50 | 17.40 | −.10 | 80.6 | 81.0 | +.4 | 3.40 | 3.30 | | |
| No. 27 | 14.55 | 18.15 | 18.27 | +.12 | 80.2 | 79.6 | −.6 | 3.60 | 3.72 | | |
| No. 28 | 15.25 | 18.65 | 18.90 | +.25 | 81.8 | 80.7 | −1.1 | 3.40 | 3.65 | | |
| No. 29 | 14.40 | 17.85 | 17.84 | −.01 | 80.7 | 80.7 | 0 | 3.45 | 3.44 | | |
| No. 30 | 14.70 | 18.20 | 18.30 | +.10 | 80.8 | 80.3 | −.5 | 3.50 | 3.60 | | |
| No. 31 | 14.85 | 18.40 | 18.34 | −.06 | 80.7 | 81.0 | +.3 | 3.55 | 3.49 | | |
| No. 32 | 14.65 | 18.00 | 18.24 | +.24 | 81.4 | 80.3 | −1.1 | 3.35 | 3.59 | | |
| No. 33 | 14.10 | 17.90 | 18.09 | +.19 | 78.8 | 77.9 | −.9 | 3.80 | 3.99 | | |
| No. 34 | 14.50 | 18.70 | 18.84 | +.14 | 77.5 | 77.0 | −.5 | 4.20 | 4.34 | | |
| Average | 14.70 | 18.23 | 18.32 | +.09 | 80.6 | 80.2 | −.4 | 3.53 | 3.62 | | |
| Maximum | 16.00 | 19.35 | 19.62 | +.42<br>−.26 | 83.3 | 82.4 | +1.2<br>−1.9 | 4.20 | 4.34 | | |
| Minimum | 13.70 | 17.30 | 17.18 | ±0<br>±0 | 77.5 | 77.0 | ±0<br>±0 | 3.05 | 3.19 | | |
| Summary: | | | | | | | | | | | |
| 109 determinations— | | | | | | | | | | | |
| Average | 13.48 | 17.18 | 17.16 | −.02 | 77.10 | 77.29 | +0.14 | 3.70 | 3.68 | | |
| Maximum | | | | +.47<br>−.76 | | | +4.00<br>−2.22 | | | | |
| Minimum | | | | ±0 | | | ±0 | | | | |
| 72 determinations [1]— | | | | | | | | | | | |
| Average | 15.61 | 18.94 | 19.05 | +.11 | 82.25 | 81.75 | −.50 | 3.33 | 3.44 | | |
| Maximum | | | | +.47<br>−.33 | | | +1.30<br>−2.20 | | | | |
| Minimum | | | | ±0 | | | ±0 | | | | |

[1] On freshly harvested beets only.

In the case of the stored Colorado beets (Table 3) the percentage of solids by refractometer is always lower than by Brix and the apparent purity correspondingly higher, the average variation being −0.28

per cent solids and +1.4 purity. In the case of the freshly harvested Utah, California, and Virginia beets (Table 3), the variations are irregular, but the averages are the reverse of those of the Colorado beets in that the percentage of solids by refractometer is higher than by Brix and the purity correspondingly lower. Thus, the average variation for Utah beets is +0.07 per cent and −0.27, for California beets +0.17 per cent and −0.8, and for Virginia beets +0.09 per cent and −0.4.

The averages for all samples are practically identical, the percentages of solids by refractometer being very slightly lower (−0.02 per cent) than by Brix and corresponding purity very slightly higher (+0.09), whereas, excluding the stored Colorado beets, in the averages for all freshly harvested beets the percentage of solids by refractometer is higher (+0.11 per cent) than by Brix and the corresponding purity lower (−0.5).

A survey of the literature shows that, in general, the percentage of solids by refractometer is lower than by Brix. The literature also shows that the results by refractometer more closely approach the true solids and ascribes the higher solids by Brix to the influence of the soluble nonsugars, in particular the inorganic solids, the average refractive index of which is about the same as the index for sugars, while their specific gravity is higher than that of the sugars.

Thus, Prinsen Geerligs (*8*) found that, in general, the calcium salts afford higher refractive indexes than sucrose, the sodium salts about equal, the potash salts lower, and that mixtures of these salts equivalent to those ordinarily occurring in sugar-plant juices afford a refractive index about equal to that of sucrose. He also found that the specific gravity of the salts is much greater than the specific gravity of sucrose, d-glucose, and d-fructose, and that while the Brix hydrometer affords correct percentages of total solids on sugar in solution it does not afford correct percentages of total solids when salts are present, the percentage of solids indicated being greater than true solids in proportion to the relative quantity of salts present.

Tolman and Smith (*12*) found that sucrose, d-glucose, and d-fructose have the same refractive index for all concentrations.

In the case of the stored Colorado beets, all of the results agree with the conclusions of numerous investigations in that the percentage of solids by refractometer is lower than by Brix, but in the case of the freshly harvested beets the average results are absolutely opposite in that the solids by refractometer are higher. However, great variation occurs in individual samples among the latter, the percentage of solids by refractometer varying from that by Brix by as much as +0.47 and −0.33 per cent. No doubt the stored beets must have lost considerable sucrose through respiration and sprouting. This is indicated by the high "approximate nonsugars" and consequently low ratio of sucrose to nonsugars, and it may be assumed that the higher solids by Brix were due to the high specific gravity of these nonsugars. In the freshly harvested beets the "approximate nonsugars" are much lower and are fairly consistent, and comparison of these figures with the total solids indicates that the erratic variations between solids by refractometer and by Brix are due not only to variations in the sucrose-nonsugar ratios but that they must be greatly influenced by the character of the nonsugars. However, this

investigation related merely to a comparison of results by the two methods and did not contemplate a study of the causes of variations. It is obvious that there is no constant relation between the percentage of total soluble solids and the purity as determined by refractometer and by Brix hydrometer and that the results by either method afford only a rough approximation of results by the other.

In the case of the Utah and California beets, the utilization of the results for total soluble solids by refractometer for the purpose of determining the approximate sucrose content was investigated in a manner very similar to that used by Komers (*4*) and Munerati and Mezzadroli (*5*). The average of the purities by refractometer (sucrose÷total solids by refractometer) was used as a constant factor, and the individual total solids by refractometer were multiplied by this factor and the result considered as the approximate percentage of sucrose. These results are given in Table 3, and it will be noted that the approximate percentage of sucrose may vary from the real percentage by as much as +1.80 and −1.14 per cent. The results and conclusions agree with those of the above-mentioned investigators in that the method affords no more than an extremely rough approximation of the percentage of sucrose. High total solids by no means indicate high sucrose, and the results are so misleading that, in the opinion of the writer, the method is worthless. Sucrose must be determined by means of a saccharimeter (polariscope).

In the analysis of the small sample of pulp from an individual beet the sucrose is determined ordinarily as percentage in the beet, and it is necessary to translate this to percentage in the juice before the purity of the juice can be calculated. The formula used is—

$$\frac{\text{Percentage of sucrose in the beet}}{100-\text{percentage of fiber in the beet}}=\text{percentage of sucrose in the juice}$$

The determination of fiber ("marc") requires so much time that it is not feasible to determine it, and incidentally in many instances the size of the sample is so small that after sucrose and total solids have been determined there does not remain sufficient material for its determination. Dry fiber in beets varies ordinarily from 4 to 5 per cent and averages about 4.5 per cent, and the use of the factor 95.5 will afford results that suffice for comparative purposes. The true fiber in situ in the beet is somewhat higher, depending upon its water of hydration, but dry-fiber figures are the ones ordinarily used. Apparent purity by refractometer is obtained by dividing the percentage of sucrose in the juice by the percentage of total soluble solids by refractometer.

TABLE 4.—*Influence of fiber upon apparent purity by refractometer*

| Per centage of sucrose in beet | Per centage of total solids in juice by refractometer | Calculated percentage of sucrose in juice, on basis of fiber in beet— | | | Apparent purity: Sucrose in juice÷solids by refractometer | | |
|---|---|---|---|---|---|---|---|
| | | 4 per cent | 4.5 per cent | 5 per cent | 4 per cent | 4.5 per cent | 5 per cent |
| 14.00 | 17.50 | 14.58 | 14.66 | 14.74 | 83.31 | 83.77 | 84.23 |
| 16.00 | 19.60 | 16.67 | 16.75 | 16.84 | 85.05 | 85.46 | 85.92 |
| 18.00 | 22.30 | 18.75 | 18.85 | 18.95 | 84.08 | 84.53 | 84.98 |
| 20.00 | 25.00 | 20.83 | 20.94 | 21.05 | 83.32 | 83.76 | 84.20 |
| Average | | | | | 83.94 | 84.38 | 84.83 |

Referring to Table 4, showing the influence of different percentages of fiber upon apparent purity, it will be seen that the variation as between 4 and 5 per cent fiber amounts to 0.9 degree. Apparent purities determined in this manner are not comparable with apparent purities as ordinarily determined by dividing the percentage of sucrose by direct polarization in the juice by Brix, as they may vary therefrom by as much as plus or minus 2 or 3 degrees, but they are comparable among themselves within a range suitable for estimating comparative purity. Indeed, there appears to be no reason why the percentage of sucrose in the beet divided by the percentage of solids by refractometer in the juice will not afford quotients that will be comparable among themselves for any given lot of beets that are in normal condition.

## SUMMARY

Total soluble solids by refractometer in the juice from very small samples of sugar-beet pulp may be determined satisfactorily by squeezing the pulp with the fingers and permitting a few drops of juice to fall directly upon the prism of the instrument. The determination may be delayed with safety up to 24 hours if the pulp is stored in air-tight containers in a refrigerator at approximately 12° C.

The percentage of total soluble solids affords such an extremely rough and very unreliable approximation of the percentage of sucrose that it should not be used for this purpose. Sucrose should be determined by means of a saccharimeter (polariscope).

A method for determining "apparent purity by refractometer" in juice from very small samples of pulp is described. The figures are not comparable with apparent purity of juice as determined by the Brix hydrometer, as they may vary therefrom by as much as plus or minus 2 or 3 degrees, but they appear to be comparable among themselves within a comparatively small range of error. It is believed that the factor affords a valuable criterion for judging the comparative quality of individual beets which, apparently, from an analytical standpoint, heretofore have been judged on a basis of sucrose content alone.

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# FURTHER STUDIES ON THE SOIL RELATIONSHIPS OF THE MOSAIC DISEASE OF WINTER WHEAT [1]

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## INTRODUCTION

Simultaneously with the experiments reported in a recent paper by the writer [3] other studies were conducted by him on additional phases of the mosaic disease of winter wheat. It already has been emphasized that the causal agent is soil borne and that the disease has its natural origin or point of attack on the underground parts of plants in the seedling stage. However, no previous experiments has been directed toward a determination of the exact place of infection, and as a consequence it was not known whether infection occurred through the roots or the crown or both.

Similarly, no information was available on the development of the disease as influenced by growing the plants in layers of infested soil at different depths, or under different arrangement in the containers. Nor had tests been made, of the effect of dilution of the infested soil with noninfested soil, of filterability or removal of the causal agent from the infested soil, or of successively increasing the period during which the plants growing in infested soil were exposed to outdoor conditions.

It seemed desirable, therefore, to conduct experiments on the several phases enumerated. It was felt that the results would be highly important and fundamental to a proper understanding of this very complex and seemingly unusual mosaic disease. The experiments, it must be emphasized, have been very preliminary in both nature and scope, and they represent therefore only a beginning of the studies in this direction. It is recognized that the results are far from being complete and final. The data, however, certainly contribute interesting information on important relations as well as suggest a complexity of problems for subsequent research.

## METHODS AND MATERIALS

The methods involved were substantially those reported recently by the writer,[4] and the details may be obtained by reference to that paper. It may be added, however, that the experiments were conducted in the outdoor plots at Madison, Wis., during the winter-

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[2] The writer is grateful to H. H. McKinney for suggestions and assistance; also to C. R. Ball and A. G. Johnson for helpful suggestions concerning the manuscript.

[3] WEBB, R. W. SOIL FACTORS INFLUENCING THE DEVELOPMENT OF THE MOSAIC DISEASE IN WINTER WHEAT. Jour. Agr. Research 35: 587–614, illus. 1927.

[4] WEBB, R. W. Op. cit.

wheat seasons of 1923–24 and 1924–25. All of the experiments reported have been conducted in season; that is, the grain was sown during the fall on a normal seeding date, and the plants experienced a normal or shortened period of dormancy. All precautions were taken to prevent winterkilling. Numerous punctures were made in the bottom of each container, and the containers were embedded in the ground up to the soil line over a specially prepared layer of gravel and cinders in order to facilitate underdrainage. Openings also were made in the containers above the soil line to promote free and rapid surface drainage, and the surface of the soil was sloped toward the openings. Upon the approach of low air and soil temperatures, a thin mulch was placed over the plants, and they generally passed the winter satisfactorily under these conditions.

In the first experiments only the Harvest Queen variety of wheat was tested, whereas in the later experiments both Harvest Queen and Currell varieties were used. It will be remembered that the first variety mentioned is one of the few excellent ones for expressing both the rosette and the mottling phases of the disease, while the second is one of the many excellent ones for demonstrating only the mottling symptoms. Hand-selected kernels of each variety were chosen, and the seeds were sown at the rate of 1 gram per linear foot in each of three rows in each container. In the experiments involving infested soil in cylinders of different diameters, a different method of seeding necessarily was adopted, as will be noted later. The grain was sown at a depth of 1 inch in the experiments of 1923 and 1.5 inches in those of 1924. The sowing dates in the two years were practically identical, ranging from September 28 to October 2 in 1923, and from September 30 to October 3 in 1924. Germination always was good and the seedlings emerged within the usual period.

The natural infested soil employed was the usual soil brought from Granite City, Ill. This soil is a fine sedimentary type, and is spoken of as a heavy gumbo. Fertile loam soil obtained in the vicinity of Madison, Wis., served as the noninfested soil component in the different stratification or dilution series and as a control in all the experiments.

Several different types of containers were used. For the most part, discarded metal containers from the soil-temperature tanks were employed. The diameters were 6, 8, or 10 inches, and the depths were 8 or 10 inches. Containers of uniform dimensions were used for each particular experiment, except in one case where exceptional depths were desired. Here containers 13 inches in depth were employed in conjunction with containers 8 inches in depth, the latter being used in most of the experiments. In the filtration experiments, where larger containers were desired, ordinary large ash pails were used. Small wooden flats were used in the experiments on dormancy. These flats were 12 by 14 by 8 inches.

The experiments were generally conducted in duplicate, and the results from the duplicate containers generally were very consistent. Where the infested soil was arranged as in cylinders of different diameters, the experiments were conducted in quadruplicate. The total number of plants from the duplicate containers, which survived the winter and on which these results were based, generally ranged from 60 to 100. In many cases, however, the total number was much greater than the range specified.

## STRATIFICATION OF INFESTED SOIL

In the attempt to gain information concerning the particular location on the seedling at which infection occurs, the method of soil stratification was employed.

### PROCEDURE

Layers of infested soil of different thicknesses were placed in the containers in various positions with respect to the position of the sown grain. In two experiments (A) in successive years, a layer of infested soil of varying thickness was placed in each of a series of containers, beginning at the bottom. In two similar experiments (B) the layer was from the top of the container downward. In one other experiment (C) a uniformly thin layer of infested soil was placed at different depths in the series of containers.

Experiments were conducted also (D) in which the grain was sown in infested soil arranged in the form of cylinders of different diameters, and surrounded by noninfested soil. In a complementary experiment the noninfested soil was arranged in the form of cylinders of different diameters and surrounded by infested soil.

In setting up the various stratification experiments, great care was exerted in order that the results might possess their fullest significance. Both the infested and noninfested soils were thoroughly screened and brought to excellent friability by adjustment to the proper moisture content. The lower layer of soil in each case was carefully placed and gently but firmly packed with a tamper of about the same diameter as the container. The next layer was placed and handled similarly, and the next, and so on. When the level for seeding was reached, the grain was sown and then covered with the desired thickness of soil. The layers of infested or noninfested soil thus were arranged with no possible disturbance by the addition of other layers, by the sowing of the grain, or by the covering of the grain.

Similar care was taken in the preparation of the experiments involving infested or noninfested soil in cylinders of different diameters. Noninfested soil to the depth of 4 inches was first placed in the containers. Then a hollow paraffined cylinder made of heavy paper and possessing the proper diameter was placed vertically in the center of the container, resting on the noninfested soil. Infested soil was then added either within or without the cylinder, according to the nature of the experiment. After the paper cylinder and the surrounding container had been completely filled with the proper soil, the cylinder was gently pulled out until the lower end was half an inch below the soil line. In this position the extending part of the cylinder was cut off so as to leave a 1-inch collar above the soil line and thus prevent diffusion of the two soils near the surface. These paraffined collars remained throughout the experiments.

### LAYERS OF INFESTED SOIL AT THE BOTTOM OF THE CONTAINERS

Experiment A was begun in 1923–24 and continued in 1924–25, with some expansion and other modification.

In the experiment of 1923–24 the layers of infested soil of different thicknesses were placed in the containers, beginning at the bottom.

Seven containers, 8 inches deep, were used for each variety, besides the control. The layers of infested soil ranged in thickness from 2 to 8 inches, being 2, 3, 4, 5, 6, 7, and 8 inches thick, respectively. The seeds were sown at a uniform depth of 1 inch, and the distances from the seeds to the top of the infested soil ranged by 1-inch stages from 5 to 0 inches, as noted in Table 1.

TABLE 1.—*Thickness and relative positions of the layers of infested and noninfested soil in the containers, their position in relation to the seeds sown, and the effect of these factors on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell, winter wheats, in 1923–24 and 1924–25, when the infested soil was beneath the noninfested*

[Mottling symbols: F=faint, C=conspicuous]

| Season | Thickness (in inches) of layer of— | | Total thickness of soil in containers (inches) | Distance from seed sown to top of layer of infested soil (inches) | | | | Disease developing in— | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Harvest Queen | | | | Currell, mottling in 1924–25 |
| | | | | Downward | | Upward | | Rosette (per cent) | | Mottling | | |
| | Infested soil placed at bottom of containers | Noninfested soil placed at top of containers | | 1923–24 | 1924–25 | 1923–24 | 1924–25 | 1923–24 | 1924–25 | 1923–24 | 1924–25 | |
| 1924 | 1.0 | 12.0 | 13 | ---- | 10.5 | ---- | ---- | ---- | 0 | ---- | F | F |
| | 2.0 | 11.0 | 13 | ---- | 9.5 | ---- | ---- | ---- | 0 | ---- | F | F |
| | 3.0 | 10.0 | 13 | ---- | 8.5 | ---- | ---- | ---- | 0 | ---- | F | F |
| | 4.0 | 9.0 | 13 | ---- | 7.5 | ---- | ---- | ---- | 0 | ---- | F | [a]F |
| | 5.0 | 8.0 | 13 | ---- | 6.5 | ---- | ---- | ---- | 0 | ---- | F | F |
| | 1.0 | 7.0 | 8 | ---- | 5.5 | ---- | ---- | ---- | 0 | ---- | F | F |
| Both years | 2.0 | 6.0 | 8 | 5 | 4.5 | ---- | ---- | 22 | 1.4 | Present. | F | F |
| | 3.0 | 5.0 | 8 | 4 | 3.5 | ---- | ---- | 33 | 2.5 | ---do--- | F | F |
| | 4.0 | 4.0 | 8 | 3 | 2.5 | ---- | ---- | 42 | 4.9 | ---do--- | F | F |
| | 5.0 | 3.0 | 8 | 2 | 1.5 | ---- | ---- | 42 | 16.9 | ---do--- | F | F |
| | 6.0 | 2.0 | 8 | 1 | .5 | ---- | ---- | 56 | 25.0 | ---do--- | F | F |
| 1924 | 6.5 | 1.5 | 8 | ---- | 0 | ---- | 0 | ---- | 28.3 | F | [b]C | |
| Both years | 7.0 | 1.0 | 8 | 0 | ---- | 0 | .5 | 55 | 70.5 | Present. | C | [b]C |
| 1924 | 7.5 | .5 | 8 | ---- | ---- | ---- | 1.0 | ---- | 80.0 | C | [b]C | |
| Both years | 8.0 | 0 | 8 | ---- | ---- | ---- | 1.5 | 90 | 85.1 | Present. | C | [b]C |
| | Control. | 8.0 | 8 | Control. | Control. | Control. | Control. | 0 | 0 | None. | O | O |

[a] 1 stunted plant.

[b] 50 per cent of the plants showed conspicuous mottling.

A similar experiment was conducted in the following year, 1924–25, in which five additional containers 13 inches deep were used. The infested soil layers of different thicknesses were removed still farther from the sown grain in the deeper cans. (Fig. 1.) The thickness of the layers of infested soil was adjusted to half-inch gradations in the region of the sown grain by interpolating two more cans in the series. The methods otherwise were the same in the two years, except that the grain was sown at a depth of 1.5 inches in 1924 instead of 1 inch as in 1923.

The results of experiment A, with infested soil in the bottom of the container, are shown in Table 1 and in Figure 1. The effects on disease development are shown in Figure 1 in two ways. The percentages of rosette in the Harvest Queen variety in both years are shown by means of curves, while the intensity of mottling in both varieties is shown by a series of letters adjacent to the diagram of the containers. In these series O means no mottling; F, faint mottling; and C, conspicuous mottling. Figure 1 shows also a

schematic illustration of the series of containers used in both years, the thickness of the layers of infested soil, the distance from the wheat seeds to the top of the infested soil, and the position of the sown seed in the two years. The seedings at a depth of 1 inch are those of 1923, and those at a depth of 1.5 inches are those of 1924.

### Effect on Rosette Development

The curves of Figure 1 are drawn on the basis of the percentage of rosette-diseased plants as related to distance of the infested soil from the seeds. There are slight and uniform deviations in the

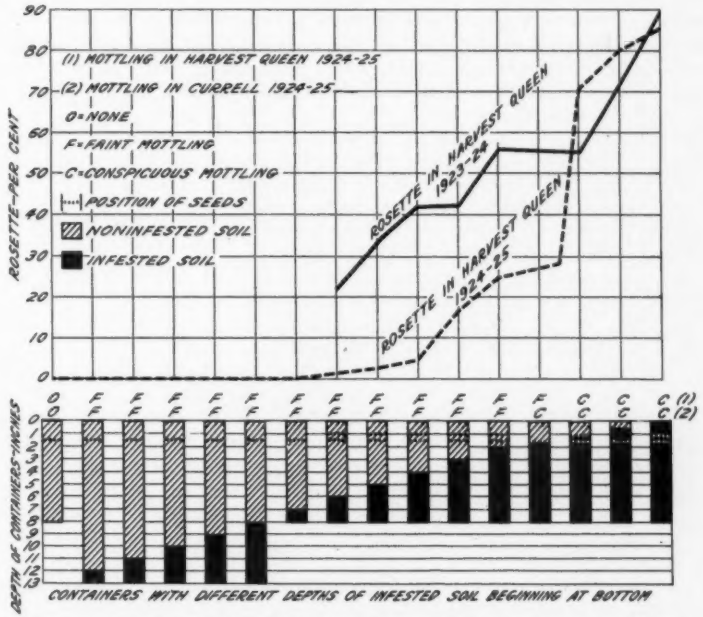


FIG. 1.—Diagram showing schematically the number and depth of containers, the position and thickness of the layers of infested soil, the position of the sown seed, the severity of mottling (by letters) of Harvest Queen in 1923–24 and of Currell in 1924–25, and the percentages of rosette (by curves) in Harvest Queen in both years with infested soil beneath the noninfested

distance between the sown grain and the infested soil of corresponding cultures in the two years. However, as the relations shown by symptom expression are very similar, and the percentages of disease in the experiment of 1923–24 were generally higher than in the corresponding experiment of 1924–25 (even though the grain was uniformly one-half inch farther from the infested soil in this case), there is justification for presenting the two curves as they are. It should be emphasized that the percentage of diseased plants at any particular point on the curves possesses relatively little importance when standing alone. It is only when each percentage is taken in conjunction with other percentages that the data assume their highest value. It is the nature and trend of the curve, therefore,

rather than any single point on the curve that really possesses significance and importance.

Examining further the results of 1923–24 in Table 1 and the corresponding curve in Figure 1, it is evident that the highest proportion of rosette-diseased plants (90 per cent) occurred in the container entirely filled, or having 8 inches of infested soil. When the layer of the infested soil was reduced to 7 inches in thickness and the grain was sown directly on the infested soil and covered with noninfested soil, the percentage diminished abruptly to 55. On reducing the thickness of the infested layer by an additional inch with a corresponding distance of 1 inch between the seeds and the infested soil, the percentage remained practically the same, namely, 56. A uniform reduction of 14 per cent in diseased plants resulted with the next two diminutions in the thickness of the layer of infested soil. In each of these containers 42 per cent of the plants showed that rosette had developed from a 5-inch and a 4-inch layer of infested soil the upper surfaces of which were 2 and 3 inches below the sown grain, respectively. A further reduction of approximately 10 per cent occurred successively in each of the next two containers of the series, the percentages of infection being 33 and 22, respectively. The latter percentage was obtained from the layer of infested soil having the least thickness (2 inches) and the greatest distance (5 inches) from the sown grain.

The effects on the rosette phase of the mosaic disease produced in the 1924–25 experiment also are tabulated in Table 1 and shown graphically in Figure 1. They demonstrate the same general relations. Although the curves from the two experiments are very similar, the percentages of diseased plants from the thin layers of infested soil considerably removed from the sown grain generally were higher in 1923–24 than in 1924–25. With greater thickness of infested soil, extending to and above the sown grain, the percentages for 1924–25 were approximately the same as, or somewhat greater than, those for 1923–24. These inconsistencies are to be expected in field studies of this type, especially when soil moisture and soil temperature play such a tremendously important part as was shown recently by Webb.[4]

The important features of the development of the rosette stage, however, are very consistent, despite these yearly variations. Again, the highest percentages of diseased plants occurred in the containers entirely filled with infested soil. There is a small but consistent decrease in the percentage of infection as the layer of infested soil above the seeds is diminished in thickness. Again, too, a sharp drop in the curve occurs when the grain is sown directly on the upper surface of the infested layer, with only the noninfested soil above the sown grain. The reduction was 35 per cent in 1923–24 and 57 per cent in 1924–25, and these values represent the maximum decline of the curve for any one uniform gradation within the experiment.

The rosette curve for 1924–25 shows that the presence of only a half-inch layer of infested soil above the grain, in addition to a 6½-inch layer beneath, produced a striking increase in percentage of rosette-diseased plants, compared with no infested soil above the seed. The plants from beneath the half-inch layer showed 70.5 per cent of rosette as compared to 28.3 per cent when sowing was on the

[4] WEBB, R. W. Op. cit.

surface of infested soil. It thus appears that considerable infection may and does take place through the crown of the seedling.

As noted, rosette occurred under the conditions of the extreme limits of the experiment in 1923–24, when 22 per cent of the plants showed disease which had developed from a 2-inch layer of infested soil placed 5 inches below the sown grain. In 1924–25 the range of the experiment was further expanded until the extreme containers involved only a 1-inch layer of infested soil placed 10.5 inches below the sown grain. Examining the curve further, it is evident that no rosette appeared in the container corresponding to the lower limit of the experiment during the previous year, nor in any of the deeper containers with infested soil at still greater distances from the seed. The rosette phase made its last appearance in the series with a 2-inch layer of infested soil placed either 4.5 or 5 inches below the sown grain.

### Effect on Development of Mottling

Thus far, only the effect on the rosette phase of the mosaic disease has been considered for the experiments of the two years. The relations concerning mottling, however, are equally as interesting. When the two sets of relations are taken together, they substantiate earlier conclusions, namely, that the rosette and the mottling expressions are different responses to the same causal agent and probably represent different degrees of severity.

From the mottling data for 1923–24 in Table 1 and the diagrammatic presentation in Figure 1, it is evident that mottling was shown by the Harvest Queen variety grown above all thicknesses of infested soil, including the thinnest layer, 2 inches, placed 5 inches below the sown grain. This is not surprising, however, inasmuch as 22 per cent of the plants developed rosette also under this set of conditions. The mottling data, however, reveal the fact that the relations representing the two disease expressions are different, especially in the range with the lesser thicknesses of infested soil placed at a considerable distance below the sown grain.

As was pointed out by the writer in a previous publication,[4] the mottling data are extremely difficult to express or represent, because the percentages of mottled plants and especially the intensity of the mottling are variable. No quantitative method of expression therefore is satisfactory, and no attempt is made to construct curves representing mottling on either a quantitative or a qualitative basis. Nevertheless, the rosette phase with decreased thicknesses of infested soil and increased distances below the sown grain diminishes considerably faster than the mottling phase. In brief, the mottled plants persist in numbers relatively greater than the rosetted plants and with relatively more intense symptoms under the conditions described.

In 1924–25 practically the same relations were obtained for the mottling of the Harvest Queen variety. The limits of the experiment were widened, and this expansion furnished a better opportunity for studying the phases in question. From these results, as shown in Table 1 and in Figure 1, it is evident that mottling was obtained in all the containers where infested soil was present. Furthermore, it is very evident that mottling may develop under conditions from which rosette does not develop.

[4] Webb, R. W. Op. cit.

In the containers where the infested soil extended from the bottom of the can to above the sown grain, the mottling was very conspicuous, intense, and general. Such mottling has been listed as "conspicuous." With the elimination of infested soil above the sown grain, the seeds being sown directly on the infested soil and covered with noninfested soil, the intensity of the mottling declined abruptly and the percentage of mottled plants diminished noticeably. From this point to the limit of the experiment the mottling was very faint. While the percentages of mottled plants decreased with diminished thicknesses of infested soil placed at successively increasing distances from the sown grain, the percentages did not decline as rapidly as did those representing rosette. The most extreme condition which showed both rosette and mottling was a 2-inch layer of infested soil placed 4.5 inches below the sown grain. This agrees well with the results obtained during the previous year. Faint mottling persisted throughout the entire range of the experiments, even with lesser thicknesses of infested soil at greater distances below the grain.

The nonrosetted plants of the Harvest Queen variety generally showed mottling less intense than the rosetted plants of the same variety or than the mottled plants of the Currell variety. The fact that faint mottling occurred even when the grain was sown at a distance of 10.5 inches above a 1-inch layer of infested soil is very striking and proves more or less definitely that infection may and does take place through the roots.

In 1924–25 the Currell variety was tested simultaneously with the Harvest Queen variety under the conditions described. The results, as shown in Table 1, are very similar to those described for the Harvest Queen variety and need not be considered in detail. Several differences should be noted. Where the infested soil extended to or above the grain, only about 50 per cent of the plants of the Currell variety showed mottling, whereas the plants of the Harvest Queen variety showed mottling more or less generally. Such variations in mottling percentages, however, have been referred to in a recent paper by Webb.[4] The abrupt change in intensity of mottling from "conspicuous" to "faint" occurred generally under the same conditions as before, namely, where the infested soil was absent above the sown grain. In the case of Harvest Queen, the abrupt change in expression of symptoms occurred when the grain was sown directly on the infested soil, whereas in the case of Currell the change did not occur until a half-inch layer of noninfested soil intervened between the infested soil and the sown grain. In this connection, the relations for both mottling and rosette of the Harvest Queen variety were very similar. These relations were consistent for both rosette and mottling of the Harvest Queen variety during each of two years and for mottling of the Currell variety during one year. It thus appears very probable that considerable infection occurs through the crown itself, or through parts so immediately adjacent that they are not detectable by this method of experimentation.

### LAYERS OF INFESTED SOIL AT THE TOP OF THE CONTAINERS

The stratification series now to be considered (experiment B) is really the reciprocal of the stratification series previously discussed and was designed to determine the effect, if any, of various thicknesses

[4] WEBB, R. W. Op. cit.

of infested soil beginning at the surface and extending downward to increasing distances. The usual methods were employed. Particular care was exerted to sow the grain at the proper depth and to cover it with the proper quantity of infested or noninfested soil or both. No mixing of the two layers resulted in seeding or covering the grain.

The results of these experiments are presented in Table 2, and such relations as can be shown possible are graphically and diagrammatically presented in Figure 2.

TABLE 2.—*Thickness and relative positions of the layers of infested and noninfested soil in the containers, their position in relation to the seeds sown, and the effect of these factors on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell winter wheats, in 1923–24 and 1924–25, when the infested soil was above the noninfested*

[Mottling symbols: F=faint, M=midmottling, C=conspicuous]

| Season | Thickness (in inches) of layer of— | | Total thickness of soil in containers (inches) | Distance from seed sown to bottom of layer of infested soil (inches) | | | | Disease developing in— | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Harvest Queen | | | | Currell, mottling in 1924–25 |
| | Infested soil placed at top of containers | Noninfested soil placed at bottom of containers | | Upward | | Downward | | Rosette (per cent) | | Mottling | | |
| | | | | 1923–24 | 1924–25 | 1923–24 | 1924–25 | 1923–24 | 1924–25 | 1923–24 | 1924–25 | |
| 1924 | 0.13 | 7.87 | 8 | | 1.37 | | | | [a]0 | | O | [b]F |
| Both years | .25 | 7.75 | 8 | 0.75 | 1.25 | | | 18.0 | [a]0 | M | O | [b]F |
| | .50 | 7.50 | 8 | .50 | 1.00 | | | 28.0 | [c]2.4 | M | F | [b]F |
| | 1.00 | 7.00 | 8 | 0 | .50 | | | 39.0 | 8.9 | M | M | [b]F |
| 1924 | 1.50 | 6.50 | 8 | | 0 | | | | 21.7 | | M | [b]F |
| Both years | 2.00 | 6.00 | 8 | | | 1.00 | 0.50 | 95.0 | 28.9 | M | M | [b]F |
| | 3.00 | 5.00 | 8 | | | 2.00 | 1.50 | 97.0 | 72.4 | M | M | [b]F |
| | 4.00 | 4.00 | 8 | | | 3.00 | 2.50 | 97.0 | 74.7 | M | M | [d]C |
| 1924 | 8.00 | 0 | 8 | | | | 6.50 | | 85.1 | | M | [d]C |
| Both years | Control. | 8.00 | 8 | Control. | Control. | Control. | Control. | 0 | 0 | O | O | O |

[a] One plant somewhat stunted but not typically rosetted; no mottling present on this plant.
[b] Faint mottling occurred but on only a small part of the plants.
[c] Plants somewhat stunted but not severely rosetted.
[d] 50 per cent of the plants showed conspicuous mottling.

EFFECT ON ROSETTE DEVELOPMENT

Considering first the rosette expression in 1923–24, it is evident that rosette was obtained throughout the range of the experiment. From the test of the thinnest layer of infested soil, namely, 0.25 inch in thickness and 0.75 inch above the sown grain, 18 per cent of the plants showed definite rosette. The percentages increased to 28 with the half-inch layer and to 39 with the 1-inch layer. In the former case the lower limit of the infested soil was half an inch above the grain, and in the latter the infested soil layer rested directly on the seeds. With the 2-inch layer in which the infested soil extended 1 inch below the seeds, there was an abrupt rise in the disease curve, the value increasing from 39 to 95 per cent. With an additional 2 inches in thickness of the infested soil, the percentages remained approximately the same, namely, 97 per cent.

The effects on rosette development in 1924–25 were very consistent with those described for 1923–24. The limits of the experiment

were expanded somewhat, and finer graduations in thickness of the layers of infested soil were made in the critical zone; that is, in the region of the sown grain. Although the results were very consistent and the disease curves are very similar, the uniformly higher percentages obtained in 1923–24 as compared with those for 1924–25 are very noteworthy. The same yearly variations, however, were noted throughout all the experiments. But, as pointed out earlier in this paper, the trend of the curve is far more important than any single point on the curve.

With the two thinnest layers of infested soil, namely, 0.13 and 0.25 of an inch in thickness placed 1.37 and 1.25 inches, respectively, above the seeds, no rosette occurred. One plant in each of these

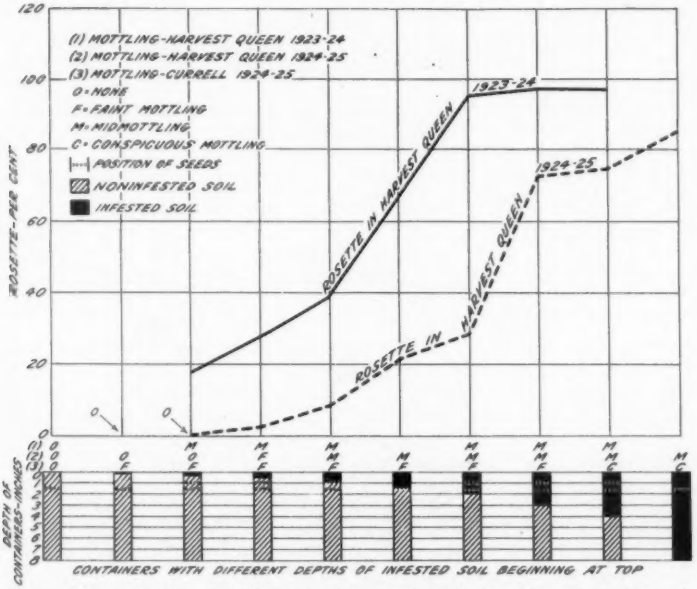


FIG. 2.—Diagram showing schematically the number and depth of containers, the position and thickness of the layers of infested soil, the position of the sown seed, the severity of mottling (by letters) of Harvest Queen in 1923–24 and of Currell in 1924–25, and the percentages of rosette (by curves) in Harvest Queen in both years, with the infested soil above the noninfested

containers presented a somewhat dwarfed but not typically rosetted condition. Furthermore, no mottling occurred. In the previous year, however, 18 per cent of rosette and midmottling occurred when the 0.25-inch layer of infested soil was used. With the 0.50-inch layer of infested soil, 2.4 per cent of the plants showed a somewhat stunted condition, but the symptoms were not those of typical rosette. Severe rosette to the extent of 8.9 per cent was obtained with the 1-inch layer of infested soil. In this case the infested soil was 0.5 of an inch above the seeds. With the 1.5-inch layer of infested soil placed directly on the grain, the percentage of rosette increased to 21.7, but with an additional 0.5 inch in thickness of the infested soil the percentage was only 28.9. However, with a 3-inch layer of infested soil which extended 1.5 inches below the sown grain, there

was an abrupt rise in the disease curve. The percentage was 72.4, an increase of 43.5, the greatest single change under the conditions of the experiments.

A similar relation was noted in the results from the experiments of the previous year, when the infested soil extended from the surface to a distance of 1 inch beneath the grain. It thus appears that infection can and does take place through both the crown and the roots, and these results agree well with those of the reciprocal experiment. Further, they demonstrate that relatively high percentages of rosette are possible only when the infested soil extends from 1 to 1.5 inches both above and below the sown grain. With increased thicknesses of infested soil beyond the above limits, the disease curve rises slowly.

### Effect on Development of Mottling

In the light of the previous results the mottling data agree very closely with what might be expected. In general, relatively higher percentages of mottling than of rosette are possible under conditions where the layers of infested soil are relatively thin and distantly removed from the seeds. In 1923–24 the mottling was of mid-intensity throughout the experiment, whereas in 1924–25 the mottling was again of medium intensity throughout except with the 0.5-inch layer of infested soil. With this, the intensity was less, and it has been graded as "faint." With further diminutions in thickness of the layers of infested soil no mottling occurred. These results are in line with the rosette data for 1924, which already have been described and contrasted with the data for 1923.

The Currell variety, when tested under similar conditions, showed mottling throughout the range of the experiment. In general, the mottling of this variety appeared more intense and conspicuous than that of the Harvest Queen variety. Several plants in each container with thicknesses of infested soil varying from 0.13 inch to 3 inches showed faint mottling. It was not until a depth of a 4-inch layer of infested soil, extending 2.5 inches below the grain, was reached that a relatively high percentage of plants showed intense mottling. With this and greater thicknesses of infested soil, 50 per cent of the plants showed conspicuous mottling. These results are very closely in line with the relations previously described in this paper.

### Half-inch strata of infested soil at different levels in the containers

The very striking results obtained from the experiments in 1923–24 suggested the importance of studying the development of the disease in relation to a uniform layer of infested soil placed at different depths with respect to the sown grain. This was done in 1924–25 (experiment C). It seemed very desirable first to employ the layer of infested soil of minimum thickness which had been found to induce a considerable production of the disease. Accordingly, a layer of infested soil of one-half inch was chosen. The experiment included such thin layers placed at half-inch intervals from the surface to 10.5 inches below the surface. As before, the experiment was conducted in duplicate, and both the Harvest Queen and Currell varieties were tested. The results are given in Table 3 and graphically shown in Figure 3, together with the schematic presentation of the position of the infested soil in the containers.

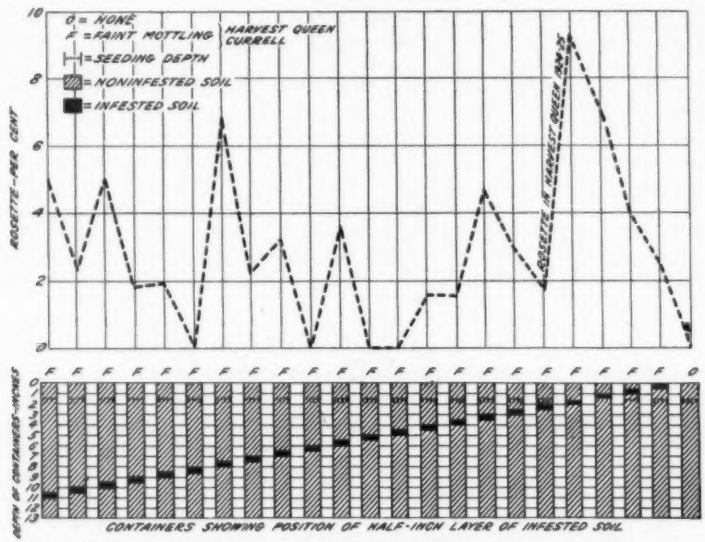


FIG. 3.—Diagram showing schematically the number and depth of the series of containers, the position of the half-inch layer of infested soil and of the seeds sown, the percentages of rosette (by curves) in the Harvest Queen variety, and the degree of mottling (F) in both the Harvest Queen and Currell varieties of winter wheat in each container, in the season of 1924–25

TABLE 3.—*Position of the half-inch layers of infested soil in the 13-inch containers, with distance from the seeds sown, and effects of these factors on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell winter wheats, in 1924–25*

[Mottling symbol: F=faint]

| Thickness of layer of noninfested soil either below or above the half-inch layer of infested soil (inches) | | Distance from seeds sown to layer of infested soil (inches) | | Disease developing in— | | |
|---|---|---|---|---|---|---|
| | | | | Harvest Queen | | Currell, mottling |
| Below | Above | Downward to top of layer | Upward to bottom of layer | Rosette (per cent) | Mottling | |
| 2.0 | 10.5 | 9.0 | ---------- | 5.0 | F | F |
| 2.5 | 10.0 | 8.5 | ---------- | 2.3 | F | F |
| 3.0 | 9.5 | 8.0 | ---------- | 5.0 | F | F |
| 3.5 | 9.0 | 7.5 | ---------- | 1.8 | F | F |
| 4.0 | 8.5 | 7.0 | ---------- | 1.9 | F | F |
| 4.5 | 8.0 | 6.5 | ---------- | 0 | F | F |
| 5.0 | 7.5 | 6.0 | ---------- | 6.7 | F | F |
| 5.5 | 7.0 | 5.5 | ---------- | 2.2 | F | F |
| 6.0 | 6.5 | 5.0 | ---------- | 3.2 | F | F |
| 6.5 | 6.0 | 4.5 | ---------- | 0 | F | F |
| 7.0 | 5.5 | 4.0 | ---------- | 3.6 | F | F |
| 7.5 | 5.0 | 3.5 | ---------- | 0 | F | F |
| 8.0 | 4.5 | 3.0 | ---------- | 0 | F | F |
| 8.5 | 4.0 | 2.5 | ---------- | 1.6 | F | F |
| 9.0 | 3.5 | 2.0 | ---------- | 1.5 | F | F |
| 9.5 | 3.0 | 1.5 | ---------- | 4.7 | F | F |
| 10.0 | 2.5 | 1.0 | ---------- | 2.9 | F | F |
| 10.5 | 2.0 | .5 | ---------- | 1.7 | F | F |
| 11.0 | 1.5 | 0 | ---------- | 9.3 | F | F |
| 11.5 | 1.0 | ---------- | 0 | 6.8 | F | F |
| 12.0 | .5 | ---------- | .5 | 4.0 | F | F |
| 12.5 | 0 | ---------- | 1.0 | 2.4 | F | F |

### Effect on Rosette Development

Very variable percentages of rosette developed when a thin layer of infested soil was placed at different depths in the containers. The proportion of rosette varied from 0 to 9.3 per cent, and the very irregular and inconsistent results were surprising. Certainly one or more factors other than the one under control in the experiments were influencing the development of the disease. Doubtless the layers of infested soil at different depths had decidedly different moisture contents and oxygen relations. The former is very important in the development of the disease, as shown recently by Webb.[4] Perhaps, also, if thicker layers of the infested soil had been placed at the different levels, more consistent effects on disease development would have resulted. It seems very evident that, under the conditions described, layers of infested soil thicker than one-half inch are essential for consistent developments of the disease. If such thin strata are employed, other influencing factors must be controlled to the highest possible degree.

Even though there was no very consistent correlation between disease development and the position of the infested soil in the experiment under consideration, several very important features are evident and substantiate previous results. In the two containers where the infested soil was directly above or below the sown grain the highest percentages of rosette resulted. When the infested soil was above the grain, rosette developed in 6.8 per cent of the plants, and where the infested soil was below the grain it developed in 9.3 per cent. It is shown again that the crown and immediately adjacent parts of the plant are very important as channels of infection. In the container where the infested soil was 6 inches below the grain, 6.7 per cent of rosette was obtained. This is the only percentage which approached those where the infested soil was in close proximity to the grain. It is surprising to note that no rosette developed in four cases, although some or all of the roots passed through the infested soil.

### Effect on Development of Mottling

Reference is made again to Table 3 and Figure 3 for a consideration of the mottling phase. The data for both the Harvest Queen and Currell varieties are very similar and will be considered jointly. In brief, mottling occurred in all the containers having the half-inch layer of infested soil, regardless of its position relative to the seeds. The mottling in all cases was very faint, and frequently it was so indistinct as to be difficult to see, even with proper shading. Only part of the plants in each of the different containers exhibited mottling. Harvest Queen showed higher percentages than Currell.

### Layers of Infested and Noninfested Soil in Cylindrical Form

In the endeavor to throw further light on the location of the point of seedling infection, a simple experiment (D) was conducted with infested soil arranged in the form of cylinders of different diameters placed in the center of the containers and surrounded by noninfested soil. The procedure followed has been considered more in detail in the description of methods. Harvest Queen wheat was sown at a

[4] Webb, R. W. Op. cit.

depth of 1 inch in the center of each central cylinder of infested soil and in the surrounding noninfested soil midway between the central cylinder and the metal container.

A reciprocal experiment also was conducted with the central cylinders of various diameters filled with noninfested soil and surrounded by infested soil. The seeds were sown as in the other case. The results obtained from these experiments are shown in Table 4.

TABLE 4.—*Development of the rosette phase of the mosaic disease in Harvest Queen winter wheat when grown in cylinders of different diameters containing infested soil and surrounded by noninfested soil, and the reverse, in 1924–25*

| Diameter of central cylinder | Cylinders containing infested soil surrounded by noninfested soil | | | Cylinders containing noninfested soil surrounded by infested soil | | |
|---|---|---|---|---|---|---|
| | Distance from seedlings in surrounding noninfested soil to cylinder of infested soil (inches) | Development of rosette on plants (per cent) | | Distance from seedlings in central noninfested soil to infested soil (inches) | Development of rosette on plants (per cent) | |
| | | Inside cylinder of infested soil | In surrounding noninfested soil | | Inside cylinder of noninfested soil | In surrounding infested soil |
| 0.5 inch | 1.40 | 50 | 3.1 | 0.25 | 100 | 98–100 |
| 1 inch | 1.25 | 50 | 0 | .50 | 100 | 98–100 |
| 2 inches | 1.00 | 100 | 15.6 | 1.00 | 100 | 98–100 |
| 3 inches | .75 | 100 | • 25.0 | 1.50 | 100 | 98–100 |
| 6 inches | | 98–100 | | | | |

• Additional 9.4 per cent of the plants showed only mottling.

In the experiment wherein the infested soil is contained in the central cylinders and surrounded by noninfested soil, it is seen that 50 per cent of the plants developed rosette when growing in cylinders of infested soil 0.5 inch or 1 inch in diameter and 4 inches in depth. When grown in cylinders of infested soil from 2 to 6 inches in diameter, 100 per cent of the plants showed rosette.

When grown in the surrounding zone of noninfested soil, the plants developed rosette more or less proportionally to their nearness to the infested soil. Very little or no rosette occurred in the containers with the least infested soil. The percentages of rosetted plants, arranged in the order of increasing diameters of the infested soil and decreasing distances from it, are as follows: 3.1, 0, 15.6, and 25. It is interesting to note that, in the last container, an additional 9.4 per cent of the plants showed mottling, making a total of 34.4 per cent of the plants showing symptoms of the disease. This is the only case where mottling developed in plants not showing rosette.

In the reciprocal experiment where the infested soil was placed outside of central cylinders of different diameters containing the noninfested soil, practically 100 per cent of the plants developed disease in both kinds of soil. The data are also presented in Table 4. At first glance it appears that the percentages of diseased plants in the noninfested soil were relatively higher in this series than when infested soil was inside. An explanation is very evident. In this second series the plants in the noninfested soil were completely surrounded by infested soil and the opportunities for infection were much greater than in the former case where the infested soil occurred on only one side of the plants.

## DILUTION OF INFESTED SOIL

The dilution method has been used effectively and continually in one way or another from almost the first recognition of the mosaic diseases, as well as of the virus diseases in general, to the present time. No research concerning such problems therefore is complete without a consideration of this procedure. The challenge with reference to the mosaic disease of winter wheat was even more urgent, inasmuch as this was the first mosaic disease recognized and proved to have a definite soil relationship. Furthermore, no previous experiments in this direction had been conducted. An opportunity thus was afforded by the dilution method for the study of symptom expression of a mosaic disease induced by a soil-infesting virus.

The infested soil was diluted in various proportions with noninfested soil. On the day before seeding, each type of soil was thoroughly pulverized and screened, united in volumetric proportions, and thoroughly mixed. The seeds were sown subsequently at a depth of 1.5 inches. The results of the different experiments are assembled in Table 5, and the curves appear in Figure 4.

TABLE 5.—*Effect of diluting infested soil with successively larger proportions of noninfested soil on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell winter wheats, in 1923–24 and 1924–25*

[Mottling symbols: F=faint, M=midmottling, C=conspicuous]

| Dilutions expressed in proportion of infested soil to noninfested soil | | Concentration of infested soil | Disease development in— | | | | |
|---|---|---|---|---|---|---|---|
| | | | Harvest Queen | | | | Currell, mottling in 1924–25 |
| | | | Rosette | | Mottling | | |
| Infested soil | Noninfested soil | | 1923–24 | 1924–25 | 1923–24 | 1924–25 | |
| | | *Per cent* | *Per cent* | *Per cent* | *Per cent* | | |
| All. | 0 | 100 | 95–98 | 88.2 | 95–98 | M | C |
| 3 | 1 | 75.0 | 47 | [a] 80.2 | 48 | M | C |
| 1 | 1 | 50.0 | 56 | 86.9 | 56 | M | C |
| 1 | 3 | 25.0 | 26 | 53.4 | 36 | M | C |
| 1 | 7 | 12.5 | 11 | 32.7 | 23 | F | [b] F |
| 1 | 15 | 6.3 | 1 | 11.0 | 6 | F | F |
| 1 | 31 | 3.1 | ---------- | 5.3 | ---------- | F | F |
| 0 | All. | Control. | 0 | 0 | 0 | None. | None. |

[a] Small bunch of escaped plants at one end of row reduced the percentage.
[b] 1 plant only showed intense mottling.

### EFFECT ON ROSETTE DEVELOPMENT

In the experiment of 1923–24, the usual high percentage of rosette developed in plants in the undiluted infested soil, and diminished percentages occurred with diluted infested soil. With concentrations of 75 and 50 per cent of infested soil, the percentages of rosette decreased rapidly and rather uniformly to 47 and 56 per cent, respectively. Further dilutions caused more or less consistent reductions in percentages of disease, until at the lowest concentration, 6.3 per cent of infested soil, only a trace, or about 1 per cent, of rosette occurred.

Examining the data from the same dilutions in 1924–25, it will be seen that the trend of the curve so obtained is more or less similar

to that for 1923–24, except in certain particulars. At the concentrations of infested soil represented by 100, 75, and 50 per cent, practically the same proportion of rosette developed, namely, 88.2 to 80.2 per cent. It is interesting to note that in this case the value for the 50 per cent dilution was approximately the same as for the undiluted infested soil, whereas in the previous year approximately a 40 per cent reduction in rosette was produced at this infested-soil concentration. It is also interesting to note that in both years the proportion of rosette in plants from the 50 per cent dilution of infested soil was 7 to 9 per cent higher than from those in the 75 per cent concentration. These small variations, however, may be coincidental. Nevertheless, they emphasize the importance of considering the entire curve and its characteristic trend rather than any single point on the curve. When the concentrations were 25 per cent and less of infested soil, the percentages of rosette decreased

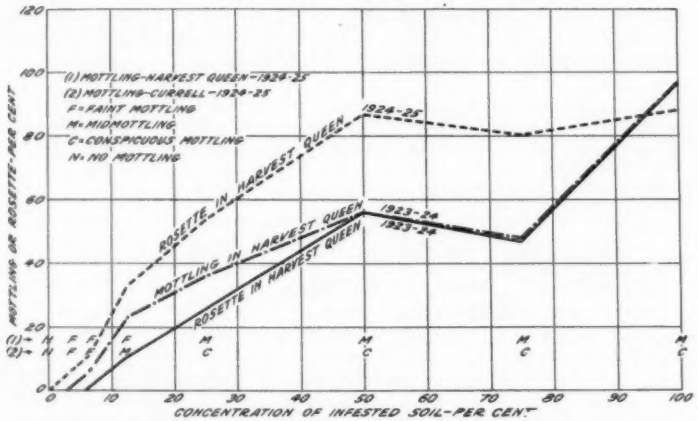


FIG. 4.—Diagram showing the effect produced on the development of the rosette and mottling phases of the mosaic disease of Harvest Queen and Currell winter wheats by diluting infested soil in different degrees by mixing with noninfested soil

rather regularly, and the part of the curve in this range is particularly similar to and consistent with that of the previous year, but the percentages in all cases are uniformly higher. The fact that 5.3 per cent of the plants developed rosette at the dilution to 3.1 per cent of infested soil is striking. However, in the previous year only a trace of rosette developed at twice this concentration.

### EFFECT ON DEVELOPMENT OF MOTTLING

The data in regard to the development of mottling in relation to the concentration of infested soil are shown also in Table 5 and Figure 4. The percentages of mottled plants were determined in the experiments of 1923–24, and they permit definite comparison, even though this method of expression is not entirely satisfactory. The curve for mottling is identical with that for rosette until the 25 per cent concentration of infested soil is reached. Here the number of mottled plants exceeds that of rosetted plants by 10 per cent, and approximately the same relation holds for the next greater dilution,

namely, that of 12.5 per cent. With the most extensive soil dilution, the proportion of mottled plants to rosetted plants is greater even than in the cases just cited. For instance, in 12.5 per cent of infested soil the ratio is 2 to 1, whereas in 6.3 per cent the ratio is 6 to 1.

Similar relations concerning mottling were obtained during the experiments of 1924–25. Mottling developed in greater quantity and intensity in the greater dilutions than did the rosette under similar conditions. There was a distinct break in the intensity of mottling at 12.5 per cent concentration of infested soil. At concentrations of 25 per cent and above, the mottling was intense, whereas at concentrations of 12.5 per cent or less the mottling was faint.

The mottling data for the Currell variety were similar to those for Harvest Queen. The mottling was very intense and very general at all concentrations of infested soil until a concentration of only 12.5 per cent was reached. Here a sharp break occurred in both the intensity and the percentage. With a concentration of 12.5 per cent or less, the mottling became very faint and was shown by only part of the plants. These data agree well with those from Harvest Queen obtained during the same and previous years.

## FILTRATION OF THE INFESTED SOIL

A simple experiment involving filtration methods was conducted during 1923–24. While the experiment was very preliminary in nature and scope, it was designed with the idea of throwing light on the physical nature of the infectious principle and of determining the applicability of such technic to the soil problems involved.

### PROCEDURE

The infested soil was thoroughly pulverized and screened, placed loosely in a large Büchner funnel over double thicknesses of cheesecloth, a considerable quantity of tap water added, and the whole filtered by suction. More water was added subsequently and drawn out. Water was added to the soil in the volumetric ratio of 2 to 1, and all possible water was drawn out of the soil. Some water, however, was retained. Considerable quantities of silt and fine particles were removed from the soil by the passage of water, and in order to remove this fraction the combined filtrates were allowed to stand overnight. The clear supernatant liquid was poured off the next day and filtered through double layers of filter paper by suction. The resulting filtrates were used as admixtures in noninfested soil.

The quantity of infested soil used in the filtration processes was twice that of the noninfested soil to which the filtrates were added. As the ratio between the water added and the infested soil was 2 to 1, the ratio between the infested soil filtered and the noninfested soil receiving the filtrates was 4 to 1. The unit quantity of noninfested soil used was half a cubic foot.

The different filtrates were applied by means of garden sprinklers to each unit quantity of noninfested soil spread out on papers in greenhouse benches. Every precaution of sterilization was taken to prevent contamination. The filtrates were added until the soil was at almost the point of saturation. The soil then was thoroughly mixed and allowed to stand. Upon standing for a day, considerable water was lost by evaporation and more of the proper filtrate was

added. These operations were repeated until the entire quantity of each filtrate had been added. The soils thus treated were stored in metal containers with metal lids and kept moist in this condition until the date of seeding, which was several weeks later.

Ash pails 11 inches in diameter and 10 inches in depth were employed as containers. The lower 5 inches of all pails was filled with the noninfested soil. The upper 4 or 5 inches in the pails was filled with the soils to which the filtrates had been added or the soils which had been filtered. In all of the previous work, infested soil 3 to 4 inches in depth has given equally as high percentages of diseased plants as greater depths of infested soil. Therefore it seemed justifiable to continue to use layers of soil of this thickness. The experiment was conducted in duplicate.

The filtrates were applied to both steam-sterilized and unsterilized noninfested soil. It seemed desirable to use sterilized soil, but in so doing there was a possibility of obtaining erroneous results, because one or more soil organisms might be instrumental in the dissemination, incubation, or inoculation of the causal agent. Thus far the available data on these points, while not conclusive, tend to be negative rather than positive. Unsterilized soil, therefore, was used to offset this possibility, but the criticism is still applicable. For instance, the infested soil came from Granite City, Ill., and contained a definite soil flora. The noninfested soil, on the other hand, came from Madison, Wis., and it also possessed a definite but probably different soil flora. All such facts should be borne in mind when studying problems of this kind.

### EFFECT ON DEVELOPMENT OF ROSETTE AND MOTTLING

The results of the filtration experiment are shown in Table 6.

TABLE 6.—*Effect of filtering infested soil, or of adding different quantities of various filtrates to sterilized and unsterilized noninfested soil, on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen winter wheat in 1924–25*

[Mottling symbols: M=midmottling, C=conspicuous]

| Infestation | Soil treatment | | | Disease developing— | |
|---|---|---|---|---|---|
| | Sterilization | Filtration | Quantity and kind of filtrates from infested soil added | Rosette | Mottling |
| | | | | *Per cent* | |
| Noninfested | Sterilized | Control | | 0 | Absent. |
| Do | Unsterilized | do | | 0 | Do. |
| Infested | do | Filtered once | | 95–98 | Present |
| Do | do | Filtered twice | | 95–98 | Do. |
| Do | do | Filtered three times. | | 100 | Do. |
| Do | do | Not filtered | | 95–98 | Do. |
| Do[a] | do | do | | 95–98 | Do. |
| Noninfested | Sterilized | | 7.75 gallons, cheesecloth filtrate | 0 | Absent. |
| Do | Unsterilized | | do | 0 | Do. |
| Do | Sterilized | | 7.75 gallons, filter-paper filtrate | 0 | Do. |
| Do | Unsterilized | | do | 0 | M.[b] |
| Do | do | | 6.25 gallons[c] of silt and dregs of all filtrates. | 25 | C.[d] |
| Do | do | | 2.50 gallons of cheesecloth filtrate from infested soil that had been filtered once. | 0 | Absent. |
| Do | do | | 6.50 gallons of cheesecloth filtrate from infested soil that had been filtered once. | 0 | Do. |
| Do | do | | 2.75 gallons of cheesecloth filtrate from infested soil that had been filtered twice. | 0 | Do. |

[a] Fresh infested soil from field.

[b] Only 2 plants showed midmottling.

[c] Dregs from 43 gallons of filtrate, the volume calculated as 0.268 cubic feet.

[d] Mottling conspicuous in all rosetted plants; a few nonrosetted plants also showed mottling.

No appreciable percentages of disease, either rosette or mottling, developed in the plants growing in soil to which filtrates from infested soil had been added. The results were negative in all cases except one, namely, where the filter-paper filtrate had been added to unsterilized soil. In this case only two plants in one of the duplicate containers showed mottling, and this would represent less than 1 per cent of the plants involved. These results are interesting in view of the fact that cheesecloth and filter-paper filtrates were added in quantities of approximately 8 gallons per 0.5 cubic foot of soil.

The silt and dregs settling out from all the filtrates were combined and the total quantity of resulting solution, 6.25 gallons, was added to a half cubic foot of soil. Calculating on a basis of the volume of particles which settled out from a known volume of filtrate upon standing overnight, it is estimated that at least 0.268 cubic foot of silt and other fine particles were added. In this soil 25 per cent of the plants developed typical rosette with very intense mottling. A few additional plants showed only mottling.

The infested soil through which large quantities of water were passed produced the disease in percentages and severity equal to that from the infested soil which had not been so treated. From the results obtained with the residues, it is evident that either relatively little or none of the virus is removed, or, if much of it is removed, it can not be detected by the methods employed. Further, the results with the filtrates indicate that little or none of the virus is removed in filtration, or, if it is removed, it soon loses its virulence. Apparently, the virus is intimately associated with the finer particles and somewhat difficult to remove from them, as is shown by the results of the filtration experiment where 25 per cent of rosette developed only upon the addition of a relatively large quantity of infested filtrate silt to the noninfested soil.

## DISCUSSION OF RESULTS

In presenting the data, it has been necessary to consider the results in more or less detail under the different headings, and this largely eliminates the necessity for discussion at this point. However, there are several features to which reference should be made.

The quantitative determination of such disease symptoms as are reported here is a difficult problem. The rosette phase is more or less constant, and it can be expressed in terms of percentages of rosetted plants. But the mottling phase, as was pointed out by the writer in a recent paper,[4] is a constantly and spasmodically changing expression in regard both to percentage and to intensity.

It is strikingly evident that the disease development, especially that of the rosette phase, varied considerably in the two different years under similar experimental conditions. The results, however, were more or less consistent throughout for each year, and this feature is interesting. In general, the percentages of rosette were decidedly higher in 1923–24 than under corresponding experimental conditions in 1924–25. The seeding dates for the two years were very nearly the same, and the soil temperatures were very similar.

On examining the soil-temperature data for the several weeks subsequent to seeding in the two different years, it is evident that in

[4] WEBB, R. W. Op. cit.

1923 the mean weekly soil temperature was 2° to 3° C. higher and that the maximum weekly soil temperature was 6° to 8° C. higher than the corresponding values for 1924. This period is a very critical one for infection and subsequent development of the disease, as was previously shown by the writer, and the variation of a few degrees in soil temperature at this temperature range and stage of plant development may explain the results. It must be remembered, however, that soil moisture also is an important factor in this connection. All the cultures of a particular series were subjected to uniform soil temperature and soil moisture conditions. Two points are important in these relations, namely, (1) that the general nature and trend of such disease curves are more important than any singel point on the curve, and (2) that too much emphasis must not be placed on the disease development under uncontrolled environmental conditions during any single year.

The results from the stratification experiments demonstrate that infection may occur through either the roots or the crown or both. Experiments of this type naturally are open to criticism, and it is not the intention of the writer to place greater value on the results than they deserve. Despite the possibilities for diffusion of soils or movement of water by either seepage or capillarity, the results are striking and suggestive.

The data at hand tend to indicate that the proportion of infection which takes place through the roots is approximately equal to, if not a little more than, that which takes place through the crown. This comparison is based chiefly on the assumption that the percentage of rosette, or symptom expression, other things being equal, is a function of the infection quantity. The following data, representing percentages of rosette, are cited as examples. In experiment A, during 1923–24, only 55 per cent of rosette developed when a layer of infested soil 6.5 inches thick was below the seeds, whereas 90 per cent developed when an additional 1-inch layer of infested soil extended above the seeds. For the similar experiment during 1924–25 the corresponding data are 28.3 and 70.5 per cent. Thus it appears that two-fifths of the rosette in 1923–24 and three-fifths in 1924–25 were caused partially or entirely by infection through the crown.

In experiment B, during 1923–24, only 39 per cent of rosette developed when a 1-inch layer of infested soil was used merely to cover the seeds, whereas 95 per cent of rosette resulted when the infested soil extended an additional inch in depth below the seeds. Corresponding data from the similar experiment during 1924–25 are 21.7 and 72.4 per cent, the latter percentage, however, resulting from a soil layer extending 1.5 inches below the seeds. Under these conditions, it appears that almost three-fifths of the rosette in 1923–24 and more than two-thirds in 1924–25 were due partially or entirely to infection through the roots. It should be stated that infested soil extending to a depth of only half an inch below the seeds increased slightly the percentage of rosette. Adding the calculated values for both crown and roots, as determined in the experiments A and B during successive years, total percentages are obtained which are surprisingly similar to the tested values for the infested-soil controls. In view of this fact, therefore, it is felt that the different calculated components representing crown and root infection, respectively, possess considerable significance.

It is of interest to note that typical rosette developed even when the top surface of the infested soil was 5 inches below the grain and that mottling developed even when the infested soil was 10.5 inches below the grain. While the cited value for rosette appears to be the limit for this expression of disease, it is very evident that the limit for the mottling phase was not reached.

In all cases where the conditions of the experiments have been such as to reduce the development of the disease, the expression of the mottling phase has persisted in higher percentages and greater intensity than the expression of the corresponding rosette phase. Only the mottling expression has appeared in certain cases. These relations further substantiate all the previous tentative conclusions, namely, that the distinctly different symptom expressions represent largely, if not entirely, different degrees of disease induced by the same causal agent.

The results obtained from dilution of the infested soil with non-infested soil are rather interesting. Experimentation of this type furnishes an excellent opportunity for study of the virus. By conducting such a dilution experiment over a period of years, the data obtained should indicate whether or not there is any change in the virus. Such an experiment would not separate the possibility of changes either in quantity or virulence or both. Nevertheless, it should yield valuable results and create suggestive leads.

The application of filtration technic to the soil problem in question introduces a complexity of problems and factors the solution of which would contribute substantially to a proper understanding of this so-called unusual mosaic disease. While the simple filtration experiment reported in this paper did not reveal any illuminating information concerning the nature of the causal agent, the results obtained under the conditions described do indicate that the causal agent is tenaciously associated with the finer soil particles. Furthermore, it would appear that the virus is more or less difficult to separate from the soil particles. However, the limited accumulation of negative results with the filtrate studies reported should not be taken as final. Additional experimentation concerning this phase is very desirable.

The infested soil, it will be remembered, is a very fine silt and belongs to the "heavy gumbo" type. The large proportion of fine particles, together with the high content of organic matter, necessarily would increase absorption and tend to make the liberation of any material, such as a virus, difficult or impossible. During the filtration process described, the soil packed and sealed in characteristic manner tightly on the cheesecloth resting in the Büchner funnel. Thus it seems very possible that the soil itself may have acted as a filter and, as such, may have retained the virus. Certainly this possibility must be considered until the results of future experimentation disprove any such relation.

If the results possess as much weight as do ordinary filtration results, it appears that the passage of large quantities of water does not remove the infectious principle. This angle of the problem has an important bearing, inasmuch as there is an open question to-day regarding the removal of the causal agent by rain water and its subsequent transportation to, and inoculation of, the soil in adjacent fields. The conditions created by heavy rains and subsequent run-

off and seepage, however, introduce differing factors. Here large quantities of water are kept in close contact with the soil particles for relatively long periods. In the filtration experiments herein reported the water was applied to the infested soil, then drawn off in a few minutes, and the operation repeated several times for each sample.

The disease developments obtained with the silt fraction of the infested soil filtrate indicate that relatively large quantities of such particles are necessary for any appreciable production of the disease. Further, the results with the infested-soil residues indicate that no detectable diminution of the disease is caused by the passage of large quantities of water, under the methods employed. Proper physical and chemical methods undoubtedly would assist materially in the successful liberation of the active agent, but there is no available evidence concerning the tolerance of the causal agent toward such treatment.

## SUMMARY

Infection of seedlings by the causal agent of the mosaic disease of winter wheat may and does occur through either the roots or the crown or both.

The disease develops when the infested soil is either below, above, or lateral to the seeds and at a considerable distance from them.

When the infested soil occurred only below the grain, from 28 to 55 per cent of the plants showed rosette; when only above, 22 to 39 per cent; and when both below and above, usually 70 to 95 per cent. The tissues of and near the crown appear highly susceptible.

A 2-inch layer of infested soil, extending 1 inch below and 1 inch above the seeds, is sufficient to cause a relatively high percentage of rosette.

With diminishing thickness of the layer of infested soil, beginning at either the top or the bottom of the containers, the percentage and intensity of disease development is correspondingly reduced, within limits.

Five inches below the seeds was the greatest distance at which infested soil caused the rosette expression. Mottling developed at the extreme limit of the experiments, namely, with infested soil at a distance of 10.5 inches below the seeds.

A layer of infested soil 0.25 of an inch thick, placed at the surface of the container (0.75 of an inch above the seeds), caused 18 per cent of rosette in the experiments of one year and none under similar conditions in the next year.

Environmental conditions exert an important influence on the development of the disease. The general nature or trend of any disease curve, therefore, is more important than any single point on the curve.

A half-inch layer of infested soil placed at each of slightly differing successive levels in the containers produced very inconsistent results. The percentages of rosette varied from 0 to 9.3, the highest percentage occurring when the infested layer was directly beneath the seeds.

Relatively high percentages of rosette were obtained when the infested soil was diluted 50 per cent with noninfested soil. With greater dilutions, the percentages decreased more or less, and at the limit of the experiment (with only 3.1 per cent of infested soil), rosette developed to the extent of only 5.3 per cent.

Filtration of the infested soil by the passage of large quantities of water through it did not remove any detectable quantities of the

virus. In all cases, negative results were obtained when the soil filtrates were mixed with noninfested soil. Positive results were obtained only from the silt fraction removed from the filtrates. The infested soil through which water had been filtered several times produced no visible diminution in disease development.

The mottling phase persisted in relatively higher percentages and greater intensity, under the conditions described, than the rosette phase. Mottling generally was present at the limits of the experiment, whereas rosette was either absent or greatly reduced at such extremes.

Both the Harvest Queen and Currell varieties showed very similar mottling relations. In general, the percentages of mottled plants were greater in the former than in the latter.

Unmistakable symptoms of rosette were obtained when the plants were exposed to outdoor conditions from a normal seeding date (September 28) until October 31. After a longer exposure, the symptom expression was greater, both in percentage and intensity.

# SMUT SUSCEPTIBILITY OF NATURALLY RESISTANT CORN WHEN ARTIFICIALLY INOCULATED[1]

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## INTRODUCTION

Certain lines of corn are resistant to natural infection by smut, *Ustilago zeae*, under field conditions. This has been shown by Jones (*6*),[3] Hayes and coauthors (*4*), Garber and Quisenberry (*3*), and Immer and Christensen (*5*), who found differences in the reaction of selfed lines of corn and their crosses to corn smut. They conclude that these differences are heritable and that by selection in self-fertilized lines it is possible to obtain lines of corn either susceptible or resistant to smut.

In the winter of 1925–26 some crosses of inbred lines of corn were tested in the greenhouse for smut resistance by the writer, using methods of inoculation slightly different from those of previous investigators (*12*), who found that lines of corn which were resistant to natural infection by smut in the field were also resistant in the greenhouse. Unexpectedly high percentages of smutted plants were obtained by the writer from a cross between naturally resistant lines of corn. In fact, the percentage of smutted plants resulting from the inoculations was about the same in the cross between the naturally resistant lines as in a cross between susceptible lines. This susceptibility of the cross between naturally resistant lines of corn led to the more detailed investigation of the problem reported in this paper.

## MATERIALS AND METHODS

Seed of selfed lines of the Garrick (C. I. 207),[4] Cuban (C. I. 218), and Boone County White (C. I. 240) varieties of corn and of crosses between them were used in both greenhouse and field experiments at the Arlington Experiment Farm, Rosslyn, Va. The seed was furnished by C. H. Kyle, agronomist in the Office of Cereal Crops and Diseases. Some of the lines had been selected for resistance and some for susceptibility to smut under field conditions at the Arlington farm.

Since a virulent culture of corn smut was desired, and since it was known that different cultures differ in pathogenicity (*8*, *12*), the smut for the inoculations was obtained from different localities. In addition to the collections from the Arlington Experiment Farm, there were cultures from California, Pennsylvania, Tennessee, and

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[2] The writer is indebted to R. S. Talbott, who assisted in making the inoculations and in taking the smut data, and to A. M. Hurd-Karrer and B. O. Dodge, who made helpful suggestions in the preparation of the manuscript.

[3] Reference is made by number (italic) to "Literature cited," p. 89.

[4] Accession number, Office of Cereal Crops and Diseases.

Texas. The history of each smut collection, so far as known, is summarized in Table 1.

TABLE 1.—*History of collections of Ustilago zeae used in experiments*

| Collection | Locality where collected | Year received | Cultured from— | Collection | Locality where collected | Year received | Cultured from— |
|---|---|---|---|---|---|---|---|
| No. 2 | Rosslyn, Va | 1922 | Gall tissue. | No. 34 | Rosslyn, Va | 1925 | Gall tissue. |
| No. 8 | State College, Pa. | 1923 | Single spore. | No. 35 | do | 1925 | Do. |
| | | | | No. 36 | do | 1925 | Do. |
| No. 28 | Tennessee | 1923 | | No. 37 | do | 1925 | Do. |
| No. 29 | California | 1923 | | No. 38 | do | 1925 | Do. |
| No. 32 | Rosslyn, Va | 1922 | Do. | No. 40–43[a] | Rosslyn, Va. (greenhouse). | 1926 | Do. |
| No. 33 | Dalhart, Tex | 1924 | Do. | | | | |

[a] These collections were from galls from smutted plants of the naturally resistant cross, Garrick F54×Cuban F79, which had been inoculated with a conidial suspension from smut collection Nos. 8, 28, 32, 33, 35, and 38.

Conidial suspensions were used for all the inoculations, and these were obtained by growing pure cultures of the various collections in carrot decoction for 6 to 10 days. These conidial suspensions were mixed just before inoculation, except in one experiment in which they were used separately.

The plants were inoculated either by injecting the conidial suspensions into the young parts with a hypodermic syringe or by pouring the suspension into the tops of the plants before they had tasseled. In some cases both methods were used on the same plant.

## RESULTS

Experiments were conducted in the greenhouse in the winter of 1925–26 and in field plots in the summer of 1926 at the Arlington Experiment Farm.

### IN THE GREENHOUSE

In the first experiment plants of three $F_1$ crosses were inoculated. One was a cross between two naturally resistant parents, the second between a resistant and a susceptible parent, and the third between two susceptible parents. The parentage of these crosses is given in Table 2. Sixty seeds of each cross had been planted about every 8 inches in rows 9 inches apart on a greenhouse bench on December 4, 1925. The plantings were made in triplicate. The air temperature was kept close to 75°–80° F., although frequent fluctuations above and below these readings occurred.

The seedlings were inoculated when 18 days old, at which time they averaged less than a foot high. The age of the plants was reckoned from the date of planting throughout the investigation. Inoculation was made with a hypodermic syringe, using a mixture of conidial suspensions from several different smut collections (Nos. 32, 33, 34, 35, and 37). A method of inoculation described by Tisdale and Johnston (*12*) was used, the needle being inserted in the plant about 2 inches above the ground and the inoculum injected until it was forced out between the folded leaves at the top of the plant. In addition a quantity of the conidial suspension was poured into the top of each plant.

From five to seven days later chlorotic areas and incipient galls had appeared. The results of this experiment are given in Table 2.

TABLE 2.—*Reaction of 18-day-old corn seedlings in the greenhouse to infection by Ustilago zeae when a suspension of conidia was injected into them about 2 inches above the ground and an additional quantity poured into the top of each*

| Variety and selfed line number | Plants naturally resistant (R) or susceptible (S) | Number of plants inoculated | Plants infected | | | |
|---|---|---|---|---|---|---|
| | | | With small galls or lesions on 1 or 2 leaves | | With severe leaf or stalk galls | |
| | | | *Number* | *Per cent* | *Number* | *Per cent* |
| Garrick F54 × Cuban F79 | R × R | 158 | 34 | 21.5 | 3 | 1.9 |
| Garrick F54 × Cuban F69 | R × S | 166 | 91 | 54.8 | 5 | 3.0 |
| Garrick F90 × Cuban F69 | S × S | 163 | 54 | 33.1 | 1 | .6 |

The data show that only 3 per cent or less of either the susceptible or the resistant crosses became severely smutted. Subsequent experiments, in which some of the smut collections were used individually for inoculations, produced high percentages of smutted plants in both resistant and susceptible lines. In addition to the severely smutted plants there were some which had either small galls or lesions on one or two leaves. The percentage of plants of the cross between resistant lines which showed these lesions or small galls was 21.5, of the cross between susceptible lines 33.1, and of the cross between susceptible and resistant lines 54.8 per cent, which indicates a difference in the resistance of the hybrids to the smut.

As the plants developed it was noted that the punctures made by the hypodermic needle 2 inches above the ground showed only in the leaves. Small galls often were present on a young leaf in the region of the needle puncture and on the tip of the next younger leaf which had not been punctured. It was obvious that the needle had not penetrated the stalk but only the tightly folded leaves. A longitudinal section through these plants showed that even at the age of 33 days (two weeks after inoculation) the apical bud was still near the level of the ground instead of 2 inches above, where the inoculations had been made. It was decided, therefore, to determine the effect of injecting the smut into the region of the apical bud. Accordingly, the severely smutted plants were removed, and the remaining plants, including those with small galls on the leaves, were reinoculated in the region of the apical bud on January 7, 1926. The needle was inserted in the plant about one-half inch from the ground, instead of 2 inches, as formerly, and the mixed conidial suspension was injected downward into the very young tissue. After 10 days, galls were appearing at the base, nodes, internodes, and on the leaves of many of these plants. The severely smutted plants were removed, and notes were made on the size, number, and location of galls on each plant.

The remaining plants, which were either smut free or had but very small galls on the leaves, were inoculated again on January 30, 1926, when about 8 weeks old, at which time the apical bud was several inches from the ground. Since the plants were larger and the internodes were elongating near the base, it was more difficult to estimate the location of the apical buds. All but four plants of each of two of the crosses became smutted as a result of this reinoculation of the tissue about the apical bud, and even these few unsmutted plants became smutted after being reinoculated on February 26, 1926.

The conidial suspensions for these three inoculations were mixtures from the same smut collections (Nos. 8, 28, 32, 33, 35, and 38). The results are shown graphically in Figure 1.

The data show that over 73 per cent of the plants of both the naturally resistant and the susceptible crosses, which were resistant to inoculation 2 inches above the ground, became smutted when the inoculum was injected about two weeks later into the very young growing tissue about one-half inch above the ground. As a result of the reinoculation of the young tissue of the remaining smut-free plants, more than 97 per cent of the original number of plants became smutted. No plant of any cross remained free from smut after a third inoculation. As all of the plants of each cross became smutted, and as the smut for these three inoculations was from the same collections, it would appear that the plants of all crosses remaining smut free after the first and second inoculations were merely smut escaping.

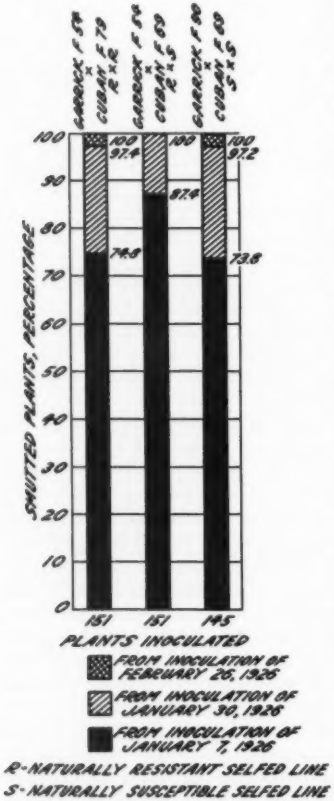


FIG. 1.—Percentages of smutted plants obtained when crosses of naturally resistant and susceptible lines of corn in the greenhouse were inoculated two or three different times by injecting conidial suspensions of *Ustilago zeae* into the region of the apical bud

Because of the unexpected susceptibility under artificial infection conditions of the naturally resistant cross, it was decided to test other lines of corn which are resistant to smut under natural conditions at the Arlington Experiment Farm. Accordingly, 40 seeds of each of five selfed lines and of a cross between two of these, all considered resistant to smut, were planted on a bench in the greenhouse on January 26, 1926. When the young seedlings were 1 month old and averaged less than a foot in height, they were inoculated about one-half inch above the ground in the young tissue about the apical bud. High percentages of plants of all the lines became smutted after this inoculation. Those plants which escaped infection were reinoculated on March 30. The two plants remaining smut free after this second inoculation became diseased after a third inoculation on April 23.

The conidial suspensions for these three inoculations were from the same smut collections as those used in the previous experiment, except that collection No. 33 was not used in the second inoculation. The data obtained from this experiment are shown in Figure 2.

After the first inoculation, 100 per cent of the plants of two of these naturally resistant lines and over 74 per cent of the plants of the other four resistant lines became smutted. After the second inoculation the percentage of smutted plants in two of these four lines rose to 100, and the percentages of smutted plants of the other two lines became 96.8 and 97.1, respectively. All of the few remain-

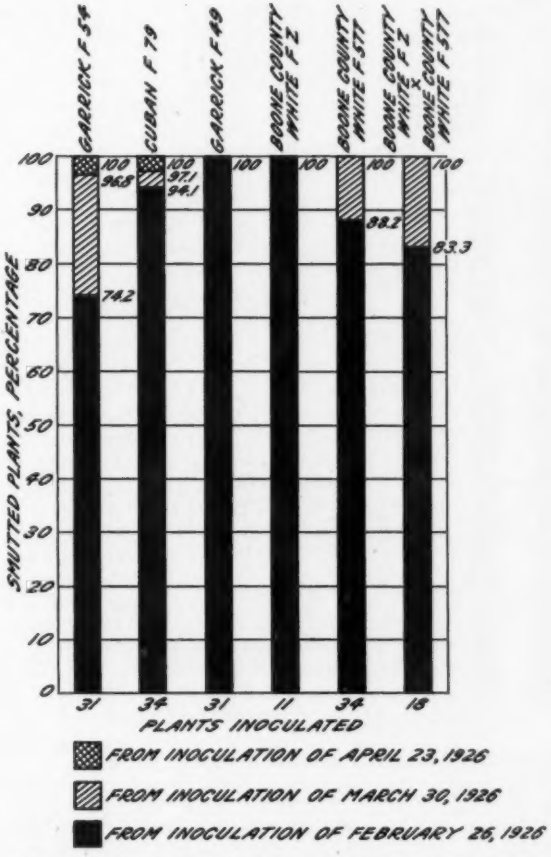


FIG. 2.—Percentages of smutted plants obtained when naturally resistant lines of corn in the greenhouse were inoculated one, two, or three different times by injecting conidial suspensions of *Ustilago zeae* into the region of the apical bud before the period of tasseling

ing smut-free plants of these lines became smutted after the third inoculation.

In order to determine whether older plants of these same six lines of corn would become smutted after injections of conidia into the very young tissues, plants of these lines about 2½ months old were inoculated on April 23, 1926. Those plants which had not tasseled were inoculated in what was estimated to be the apical region of

the stalk. In plants which had tasseled the conidial suspension was injected through the leaf sheaths in attempts to inoculate the young bud tissues at the nodes. The percentages of smutted plants resulting from this inoculation ranged from 42.9 to 94.4. (Fig. 3.) A reinoculation of those plants which had escaped infection from the first inoculation was made on May 19, 1926. All plants with the exception of a few dwarfed or stunted ones had tasseled, and the inoculum was injected into the ears and young growths from the nodes.

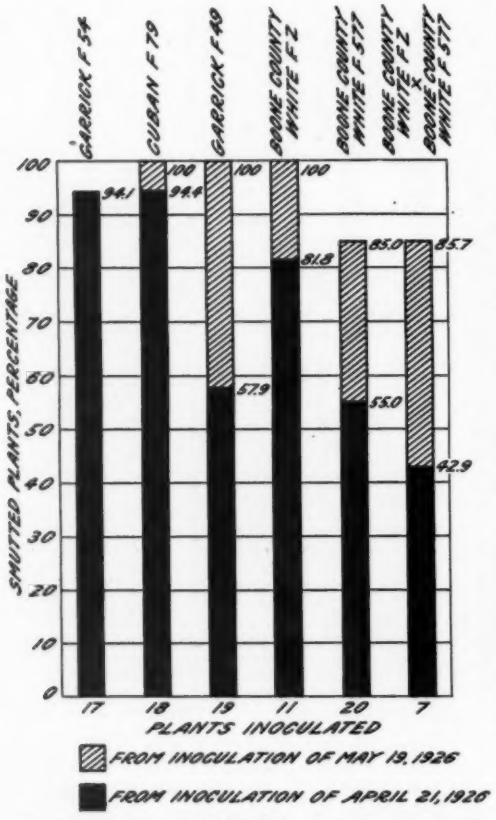


FIG. 3.—Percentages of smutted plants obtained when naturally resistant lines of corn in the greenhouse were inoculated one or two different times by injecting conidial suspensions of *Ustilago zeae* into the young tissues at about the period of tasseling

The conidial suspensions for these two inoculations were from the same smut collections used previously (Nos. 8, 28, 32, 33, 35, and 38). The results of the inoculations are shown in Figure 3.

The two inoculations resulted in 100 per cent of the plants of three lines becoming smutted, while the lowest final percentage of diseased plants in any line was 85. Evidently the age of the plant does not affect its susceptibility to smut except in so far as the development and growth of new tissue are concerned.

### IN THE FIELD

It was thought possible that the very high percentages of smutted plants resulting from the inoculation of naturally resistant lines of corn might be due to abnormal growth conditions in the greenhouse, and that in the field the plants might be resistant even if the inoculum were injected into the region of meristematic tissues. Accordingly, seeds of some of these same and other lines were planted in the field on May 20, 1926.

### Injection Experiments

On July 2, when the plants were about 2 feet high, the plants of one row of each line were inoculated with a hypodermic needle as in the greenhouse experiments, using a mixture of smut collection Nos. 8, 33, 38, and 40 to 43. In order to place the inoculum in the region of the apical bud, the plants were inoculated about an inch above the ground. At the time of these inoculations smut galls had appeared on some of the plants as the result of natural infection. One row of plants of each strain was left uninoculated to serve as a check against naturally occurring infections.

Definite signs of infection as a result of the inoculations were noted in about six days. Later, the smutted plants in both the inoculated rows and the uninoculated control rows were removed. Plants with only small galls on one or two leaves were tagged but were not removed. They were counted as smut-free plants, as it was impossible to determine whether or not these slight infections resulted from the artificial inoculation. A second inoculation of the remaining smut-free plants was made on August 6, 1926, using a mixture of smut collection Nos. 29, 32, 33, 35, 36, 37, and 38. The plants at this time had not tasseled, except those of Garrick F54 × Cuban F79. Accordingly, the injections were made near the top of the plant in the youngest tissue. The location of this tissue had to be carefully estimated for each individual plant, as it naturally varied with the stage of development. As the plants of Garrick F54 × Cuban F79 had tasseled, the injections were made in the young ears and shoots only. The percentages of smutted plants are based on the total number of plants which became smutted up to and including each inoculation date. The smut percentages resulting from the two inoculations are shown in Figure 4, together with percentages of smutted plants appearing in the control rows in each of the same two periods.

The data in Figure 4 show that more than 70 per cent of the plants of every line except one were smutted as the result of the first inoculation. The one exception was Garrick F54, which had only 30.2 per cent smutted. This comparatively low percentage is interesting because the data in Figures 2 and 3 from previous experiments and the data in Tables 3 and 4 show relatively high percentages of smutted plants in this line.

The highest percentage of smutted plants in any of the uninoculated control rows of resistant lines for this period (July 2 to August 6) was 3.6, whereas in the corresponding uninoculated control rows of the susceptible lines, Garrick F90 × Cuban F69 and Cuban F69, smut percentages of 85.7 and 74.2 per cent, respectively, were obtained during the same period.

The final percentages of smutted plants resulting from the two inoculations were high in all the lines of corn tested. The percentages of smutted plants of the five naturally resistant lines totaled 100, 93.1, 91.5, 100, and 77.4 per cent, respectively. In the uninoculated control rows of these same lines the percentages of smutted plants were 5.3, 1.7, 18.0 41.8, and 4.7 per cent, respectively. That there was no lack of opportunity for natural infection was shown by the very high percentages, 100 and 98.4, of smutted plants in the two uninoculated susceptible lines. Therefore, the high percentages of

smutted plants of these naturally resistant lines seem to have resulted from the method of inoculation, the essential element of which is evidently the injection of the smut conidia directly into the very young growing tissue.

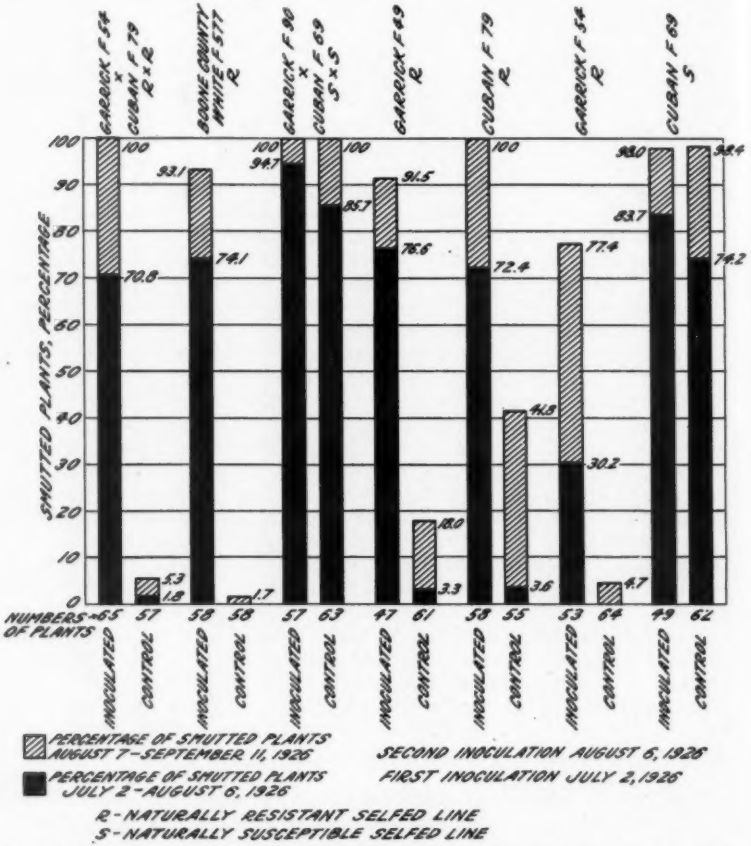


FIG. 4.—Percentages of smutted plants obtained in the field from naturally resistant and susceptible lines of corn when conidial suspensions of *Ustilago zeae* were injected two different times into very young tissue; together with percentages of smutted plants in the uninoculated controls

### POURING AND INJECTION EXPERIMENTS

For the purpose of comparing the percentages of smut obtainable by the method of injecting the conidia directly into the meristematic tissue with those resulting from the commonly used method of pouring the conidial suspension into the top of the plant, young plants about 6 weeks old were inoculated, both methods being used. Inoculum from the same mixed conidial suspension, consisting of collection Nos. 29, 32, 33, 35, 36, 37, and 38, was used in each case. In about half of the plants the inoculum was injected into the stalk

about 1 inch above the ground, which was estimated to be the approximate location of the young growing point in plants of this age. In the others the plants were inoculated by pouring the conidial suspension into the top. At this date a few of the plants had large galls on the crown, nodes, and leaves, and these were discarded. The results of the inoculations are given in Table 3.

TABLE 3.—*Comparative smut reaction in the field of plants of the same lines of corn when inoculated either by injecting a conidial suspension of Ustilago zeae into the apical bud tissue or by pouring the suspension into the top of the plant*

| Variety and selfed line number | Plants naturally resistant (R) or susceptible (S) | Plants inoculated by— | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Injecting inoculum into young tissue | | | | | Pouring inoculum into top of plant | | | | |
| | | Total | Number smutted on— | | | Percentage smutted | Total | Number smutted on— | | | Percentage smutted |
| | | | Leaf only | Stalk only | Leaf and stalk | | | Leaf only | Stalk only | Leaf and stalk | |
| Garrick F54 × Cuban F79 | R × R | 34 | 0 | 1 | 33 | 100 | 32 | 0 | 3 | 0 | 9.4 |
| Boone County White F577 | R | 27 | 2 | 2 | 20 | 88.8 | 21 | 0 | 0 | 0 | 0 |
| Garrick F90 × Cuban F69 | S × S | 31 | 1 | 2 | 28 | 100 | 26 | 0 | 23 | 1 | 92.3 |
| Garrick F49 | R | 27 | 1 | 3 | 16 | 74.1 | 25 | 0 | 1 | 0 | 4.0 |
| Cuban F79 | R | 26 | 3 | 6 | 13 | 84.6 | 24 | 0 | 1 | 0 | 4.2 |
| Garrick F54 | R | 30 | 4 | 1 | 12 | 56.7 | 30 | 0 | 0 | 0 | 0 |
| Cuban F69 | S | 34 | 3 | 6 | 21 | 88.2 | 28 | 1 | 13 | 0 | 50.0 |

The lowest percentage of smutted plants in any of the five naturally resistant lines which were inoculated by injection of the spores into the young tissue was 56.7, while in one line all of the plants were smutted. One susceptible line had 88.2 and the other had 100 per cent of plants smutted.

The highest percentage of smutted plants of any naturally resistant line inoculated by pouring the inoculum in the top was 9.4, while two lines did not show any smutted plants. The corresponding percentages of smutted plants of the susceptible lines were 50 and 92.3.

Many of the susceptible plants inoculated by pouring spores into their tops were not smutted as a direct result of the artificial inoculation but as the result of natural infection, by which many of the buds at the lower nodes later became smutted. However, it was possible to determine that the plants inoculated by injection were smutted as a direct result of the inoculation, because the smut appeared only in the parts injected and within a short time. Since the same lines of corn were used and the smut in these inoculations was from the same mixed conidial suspension, the difference in the percentages of smutted plants obviously was due to the difference in the methods of inoculation.

### INJECTIONS WITH LOCAL INOCULUM

The purpose of this investigation was to produce smut in naturally resistant lines of corn, yet in view of reports of the existence of physiologic forms of corn smut (*8*, *11*), it seemed possible that the almost

complete susceptibility of the naturally resistant lines under artificial conditions might have resulted from the use of smut from localities other than the Arlington Experiment Farm. Accordingly, another series of inoculations was made with smut collected only at the Arlington farm, consisting of collection Nos. 2, 32, 34, 35, 37, and 38. As the plants available for these inoculations had tasseled, the inoculum was injected into the ears only. The ears were in different stages of development, some with the silk just beginning to dry and others in which the silk had not extruded from the husks. The row of each of the seven lines of corn was divided into seven sections. One section was inoculated with each of the six smut collections named above, and one was left as a control. Only plants showing smut in the ears were considered smutted as the result of the inoculations. The results, as well as the order of the six inoculations, are given in Table 4.

TABLE 4.—*Number of smutted plants in naturally resistant or susceptible lines of corn in the field when conidial suspensions of six different collections of Ustilago zeae from the Arlington Experiment Farm were injected into the ears*

| Variety and selfed line number | Plants naturally resistant (R) or susceptible (S) | Uninoculated control plants | | Plants inoculated with— | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Collection No. 38 | | Collection No. 2 | | Collection No. 37 | | Collection No. 35 | | Collection No. 34 | | Collection No. 32 | |
| | | Total | Smutted | Number inoculated | Number smutted | Number inoculated | Number smutted | Number inoculated | Number smutted | Number inoculated | Number smutted | Number inoculated | Number smutted | Number inoculated | Number smutted |
| Garrick F54 × Cuban F79 | R × R | 7 | 0 | 18 | 11 | 15 | 10 | 8 | 4 | 6 | 1 | 5 | 5 | 7 | 0 |
| Boone County White F577 | R | 4 | 0 | 17 | 14 | 7 | 7 | 6 | 5 | 6 | 3 | 7 | 6 | 6 | 0 |
| Garrick F90 × Cuban F69 | S × S | 2 | 0 | 3 | 3 | 3 | 3 | 1 | 1 | 2 | 1 | 2 | 2 | 4 | 0 |
| Garrick F49 | R | 6 | 0 | 8 | 8 | 10 | 9 | 6 | 6 | 3 | 3 | 4 | 3 | 2 | 0 |
| Cuban F79 | R | 5 | 0 | 4 | 4 | 9 | 9 | 2 | 2 | 3 | 1 | 7 | 6 | 5 | 0 |
| Garrick F54 | R | 4 | 0 | 7 | 7 | 8 | 8 | 7 | 7 | 4 | 3 | 3 | 3 | 6 | 0 |
| Cuban F69 | S | 2 | 0 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 5 | 0 |

The data in Table 4 show that the lowest percentages of smutted plants in any line except one were produced in the cross, Garrick F54 × Cuban F79, in which the ears were most mature at the time of inoculation. Three of the five naturally resistant lines inoculated with collection No. 2 had every plant smutted, one had 90, and the remaining one had 66.7 per cent smutted. Collection No. 2 was obtained from the Arlington Experiment Farm in 1922 and has been grown in culture since that year.

Differences in virulence of the different smut cultures were evidenced by the time of appearance of the galls, their size, and the extent of the invasion of the host tissue of the same line of corn. Collection No. 32 did not produce any smut even in the plants of the susceptible lines. The culture of smut for this inoculation grew normally in the carrot decoction. Collection No. 34 produced galls about two days earlier than the others. Collection No. 35 produced the smallest galls and the lowest percentage of smutted plants.

Although the probable importance of physiologic strains of corn smut from different localities in any corn-breeding program is recognized, it should be emphasized that the six different collections of smut from the rather localized area of the Arlington Experiment Farm showed differences in culture and in pathogenicity.

The susceptibility of these naturally resistant lines to artificial infection when inoculated with several collections of smut from the Arlington farm indicates that the high percentages of smut obtained throughout this investigation probably are not due to the fact that collections of smut from other localities were used in the inoculations, but rather to the fact that the inoculum was injected into very young tissue. Meristematic tissue of the lines tested is susceptible to smut even in plants of lines which are highly resistant to natural infection at the Arlington Experiment Farm.

## DISCUSSION

In these experiments there apparently was little, if any, resistance of the very young host tissue to the smut. However, it is possible, of course, that there are physiological differences in the older tissues of the different lines of corn, which may determine their relative degree of resistance. Yet, in view of the fact that even in susceptible lines the galls are produced largely in the younger growing tissues, it is conceivable that resistance or susceptibility is largely a matter of relative accessibility of the susceptible parts to the invading organisms.

As has been shown, lines of corn developed at the Arlington Experiment Farm which were there resistant to natural smut infection in the field were extremely susceptible, under artificial inoculation, to several individual smut collections from the Arlington farm, as well as to mixed collections from different localities. These lines of corn as grown in the field at the Arlington farm in 1926 were resistant to natural infection by the smut occurring naturally there. (Fig. 4.) As stated previously, the naturally resistant lines used in these investigations were developed from only three varieties of corn. Consequently it is possible that the genetic factors involved in the selection of the various lines for resistance and susceptibility were limited.

If physiological differences in young tissue of different lines of corn are not determining factors in smut resistance, it is obvious that the injection method used in these experiments should not be employed for practical tests of the susceptibility of lines of corn to smut infection.

Recently Christensen and Stakman (*1*) have obtained differences in pathogenicity of physiologic forms of corn smut from different localities. Some of these forms were less virulent and some more virulent than the form from St. Paul, Minn., with which they were compared. These differences in pathogenicity perhaps would explain the susceptibility of the naturally resistant lines of corn developed at the Arlington Experiment Farm when inoculated with smuts from different localities, including the Arlington Experiment Farm. Yet even the existence of these forms of different pathogenicity from different localities does not explain the high percentages of smutted plants obtained in naturally resistant lines when several individual

smut collections from the Arlington Experiment Farm were used for the inoculations. (Table 4.)

It is possible that collections of smut from different localities would represent different physiologic forms. Yet even the different collections from the Arlington farm differed in infective power. Preliminary data concerning the pathogenicity of single-chlamydospore cultures of corn smut from the Arlington farm indicate that they differ even when obtained from the same smut gall.

It seems possible that some of the physiologic strains of *Ustilago zeae* may be due to crossing within the species. Sartoris (*9*) reported the conjugation of the conidia of *U. zeae* under certain growth conditions. It is not known whether these forms resulting from conjugation are pathogenic.

The significance of conjugation and hybridization in relation to physiologic forms of various species of fungi is apparent. Recent researches on smuts and other fungi indicate the advisability of using single chlamydospores, or even single conidia, of corn smut as the basis for determining physiologic forms of the organism. Kniep (*7*) has described and illustrated the conjugation between the germ tubes of sporidia of different species of Ustilago and the growth following these unions. Dickinson (*2*) recorded the occurrence of hyphal fusion within and across the two species of smut, *U. levis* and *U. hordei*. The pathogenicity of these new forms has not been demonstrated. Shear and Dodge (*10*) found that crossing the different species of the *Monilia sitophila* group by mating their haplonts resulted in the formation of what appeared to be hybrid perithecia with mature ascospores.

## SUMMARY

Plants of selfed lines of corn and crosses between them which are resistant to natural infection by smut in the field at the Arlington Experiment Farm, Rosslyn, Va., were very susceptible when artificially inoculated in very young tissue. The apical and nodal bud tissues, immature ears, young leaves, and tassels were all highly susceptible when injected with conidial suspensions.

These lines were susceptible to several individual smut collections from the Arlington Experiment Farm as well as to mixed collections from there and from other localities. This fact indicates that the susceptibility of the naturally resistant lines need not be attributed to the use of physiologic forms from other localities.

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# PRELIMINARY REPORT ON PARTIAL MEASUREMENTS OF FOREST PLANTATIONS [1]

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## INTRODUCTION

The measurement of forest plantations is a problem which has not as yet presented much difficulty in this country. However, as these plantations increase in area and the trees become larger, accurate measuring of the total volume will become more difficult. Adding to this the necessity of measuring such a stand periodically, it becomes essential that some method of accurately determining the total volume without actually measuring every tree be developed.

Forest plantations have certain characteristics that set them apart from native stands when the problem of volume mensuration is considered. One of the chief of these is the uniformity of plantations. The trees are ordinarily planted in evenly spaced rows, and as a rule are regularly spaced in the rows. Plantations are usually even-aged and are composed of a limited number of species. These factors produce a degree of uniformity which is not attained in a native stand.

Growth studies in a plantation must be accurate if results are to be obtained which will be dependable in comparing the effect of spacing, pruning, etc., in plantings which are being given different treatment. In measuring a portion of a stand or "sampling,"[2] there is an error introduced when this portion is taken as representative of the whole. Returning to the same stand and measuring a different set of trees (although the same percentage of the whole) would probably give a different mean due to the variation in the individual trees. How, then, can the estimator be sure that the difference found between the two stands in question is not due to this error in sampling, and that the two stands are not actually equal in volume?

It goes without saying that measuring 50 per cent of the stand would give a mean which in most cases would be closer to the true mean than measuring 25 per cent or 10 per cent of the stand. And, likewise, 75 per cent would give better results than 50 per cent. But because of the difficulty of measuring a plantation—which is often greater than that of measuring a native stand—only as small a portion as is compatible with the desired degree of accuracy need be measured. If 50 per cent of the stand will give results which are practically as good as 100 per cent, then it is not necessary to measure more than 50 per cent, and if 25 per cent will suffice in a very uniform stand it is a waste of time to cover a larger percentage of the stand.

[1] Received for publication Sept. 14, 1927; issued February, 1928.

[2] When measuring a portion of a stand it is essential that the sample be representative of the whole area and not merely chosen at random from any portion of the area. This is a basic principle in all methods requiring statistical treatment.

It is essential that the estimator determine the limit of accuracy desired before entering the stand. If an error of plus or minus 10 per cent of the total volume is allowable, a smaller percentage of the stand may be measured than if greater accuracy is desired.

A study was made in forest plantations in Ohio to determine the percentage of a stand which it is necessary to measure in order to obtain sufficiently accurate results. This study was made chiefly in white pine plantations of various spacings, but also included plantations of red pine, Scotch pine, white ash, and red oak. The stands were located at Oberlin in the northern part of the State, at Wooster in the central, and at Athens in the southern part.

## EXPERIMENTAL METHODS

The method of study was as follows: Each tree in the stand was measured for height and diameter, and a separate tally kept for each row. The averages of height and diameter were found for each stand, and the averages were then computed for various percentages of each stand by taking a certain number of rows and computing the averages for the trees in these rows. For instance, the average of 50 per cent of the stand was found by using the trees in alternate rows, and of 25 per cent by using every fourth row, etc. This method was purely mechanical in application, thus eliminating the personal factor of judgment.

The standard deviations of the two variants, height and diameter, were computed for the different percentages of the stand, and from the standard deviation the probable error was determined for each percentage. A hypothetical case (Table 1) illustrates the method used:

TABLE 1.—*Standard deviations and probable errors of heights and diameters*

| Tree No. | Height | Deviation from assumed means | Deviation squared | Diameter | Deviation from assumed means | Deviation squared |
|---|---|---|---|---|---|---|
| | *Feet* | | | *Inches* | | |
| 1 | 21.5 | 3.5 | 12.25 | 2.3 | 0.2 | 0.04 |
| 2 | 26.0 | 1.0 | 1.00 | 2.7 | .2 | .04 |
| 3 | 23.0 | 2.0 | 4.00 | 2.4 | .1 | .01 |
| 4 | 22.5 | 2.5 | 6.25 | 2.3 | .2 | .04 |
| 5 | 29.0 | 4.0 | 16.00 | 3.0 | .5 | .25 |
| 6 | 27.0 | 2.0 | 4.00 | 2.7 | .2 | .04 |
| 7 | 26.5 | 1.5 | 2.25 | 2.5 | .0 | .00 |
| 8 | 30.0 | 5.0 | 25.00 | 3.1 | .6 | .36 |
| 9 | 23.5 | 1.5 | 2.25 | 2.2 | .3 | .09 |
| 10 | 24.0 | 1.0 | 1.00 | 2.5 | .0 | .00 |
| 11 | 22.0 | 3.0 | 9.00 | 2.2 | .3 | .09 |
| Total | 275.0 | | 83.00 | 27.90 | | .56 |
| Average | 25.0 | | | 2.54 | | |

Assumed mean height=25.0 feet.
Assumed mean of diameter=2.5 inches.

$$\text{Standard deviation}^3 = \sqrt{\frac{\Sigma d^2 - na^2}{n}} \quad \text{when—}$$

$\Sigma d^2$ = the sum of the squares of the deviations (from the assumed mean).
$a$ = difference between the assumed mean and the computed mean.
$n$ = number of trees.

$$\text{Standard deviation of height} = \sqrt{\frac{83 - 11 \times 0}{11}} = \sqrt{6.55} = 2.55 \text{ feet.}$$

$$\text{Standard deviation of diameter} = \sqrt{\frac{.96 - 11 \times .04^2}{11}} = \sqrt{.08} = .28 \text{ inch.}$$

$$\text{Probable error (P. E.)} = .6745 \frac{\text{Standard deviation.}^4}{\sqrt{n}}$$

$$\text{Probable error of height} = .6745 \frac{2.55}{\sqrt{n}} = .52 \text{ foot.}$$

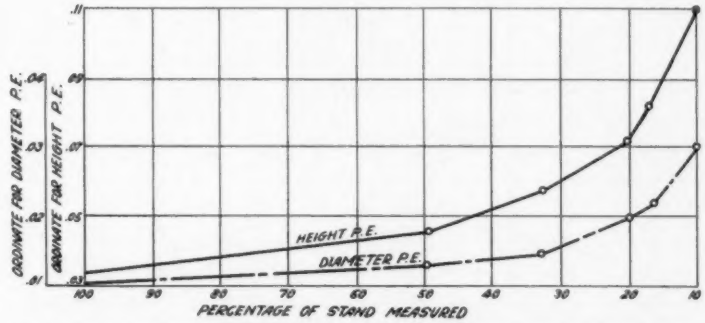



FIG. 1.—Curves for red pine plot, spacing of trees 3 × 3 feet, showing the probable error of the mean of the heights and diameters

$$\text{Probable error of diameter} = .6745 \frac{.2}{\sqrt{11}} = .041 \text{ inch.}$$

The probable error indicates the degree of accuracy attained. To illustrate: The chance of a deviation from the computed mean being greater than the probable error is equal to the chance that it will be less, and the chances are about 22 to 1 that the deviation will not exceed three times the probable error. (Differences between two means which are further apart than three times the probable error [5] have been accepted by the majority of biometricians as being significant and not due to errors in sampling.)

[3] PHILLIPS, F. M. SHORT METHOD OF OBTAINING A PEARSON COEFFICIENT OF CORRELATION, AND OTHER SHORT STATISTICAL PROCESSES. U. S. Mo. Weather Rev. 50: 135-136. 1922.
[4] YULE, G. U. AN INTRODUCTION TO THE THEORY OF STATISTICS. Ed. 7, rev., 415 p., illus. London. 1924.
[5] In this case the probable error used is the probable error of the difference, or:

$$(\text{Probable error})_{a-b} = \sqrt{(\text{P.E.})_a^2 + (\text{P.E.})_b^2}$$

## EXPERIMENTAL RESULTS

The results of the study in the stands are summarized in Tables 2 to 5. Space does not permit the printing of the curves for each plot, but those for a fairly representative plot are shown to demonstrate the trend of the probable error as diminishing percentages of the stand are measured.

TABLE 2.—*Probable error of mean of diameter (in inches)*

| Plot | Spacing of trees | Percentage of stand measured | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 80 | 66 | 50 | 44 | 33 | 25 | 20 | 10 |
| | *Feet* | *Inch* | *Inch* | *Inch* | *Inch* | *Inch* | *Inch* | *Inch* | *Inch* | *Inch* |
| White pine | 4 by 4 | 0.013 | | | 0.017 | | 0.020 | 0.031 | | |
| Do | 3 by 6 | .030 | | | .040 | | .057 | .065 | | |
| Do | 6 by 6 | .078 | | 0.092 | | 0.110 | | .160 | | |
| Do | 6 by 12 | .052 | 0.060 | | .075 | | .100 | | | |
| Do | 3 by 3 | .056 | | .070 | .075 | | .120 | .120 | | |
| Do | 3 by 3 | .020 | | | .028 | | .032 | .037 | | |
| Scotch pine | 3 by 6 | .030 | | | .037 | | .047 | | 0.062 | |
| Do | 3 by 3 | .028 | | .032 | .049 | | .051 | .052 | | |
| Red pine | 3 by 3 | .010 | | | .013 | | .015 | | .020 | 0.030 |
| Red oak | 6 by 6 | .034 | .042 | | .042 | | | .060 | | .065 |
| White ash | 4 by 4 | .052 | | | | .076 | .092 | | .108 | |

TABLE 3.—*Probable error of mean of height (in feet)*

| Plot | Spacing of trees | Percentage of stand measured | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 80 | 66 | 50 | 44 | 33 | 25 | 20 | 10 |
| | *Feet* | *Foot* | *Foot* | *Foot* | *Foot* | *Foot* | *Foot* | *Foot* | *Foot* | *Foot* |
| White pine | 4 by 4 | 0.067 | | | 0.092 | | 0.120 | 0.17 | | |
| Do | 3 by 6 | .140 | | | .190 | | .250 | .28 | | |
| Do | 6-by 6 | .180 | | 0.26 | | 0.23 | | .30 | | |
| Do | 6 by 12 | .140 | 0.16 | | .200 | | .340 | | | |
| Do | 3 by 3 | .150 | | .17 | .220 | | .250 | .31 | | |
| Do | 3 by 3 | .090 | | | .130 | | .170 | .19 | | |
| Scotch pine | 3 by 6 | .090 | | | .120 | | .160 | | 0.200 | |
| Do | 3 by 3 | .075 | | .09 | .110 | | .140 | .16 | | |
| Red pine | 3 by 3 | .034 | | | .046 | | .058 | | .072 | 0.11 |
| Red oak | 6 by 6 | .170 | .19 | | .240 | | | .35 | | .48 |
| White ash | 4 by 4 | .380 | | | | .45 | .480 | | .610 | |

The limit of accuracy to be desired in forest plantations will vary somewhat, but, for the most part, variation in results should not greatly exceed the degree of accuracy in the field work, viz, $\pm 0.1$ inch in the mean of diameter and $\pm 0.5$ foot in the mean of height. With these figures in mind the above results may be analyzed and the percentage of each stand which it would have been necessary to measure to obtain this degree of accuracy calculated. Since the true mean will usually (22 times out of 23) be found within the limits of plus or minus three times the probable error, the desired accuracy restricts the probable error to one-third the limits decided upon, or to approximately 0.2 foot in height and 0.04 inch in diameter.

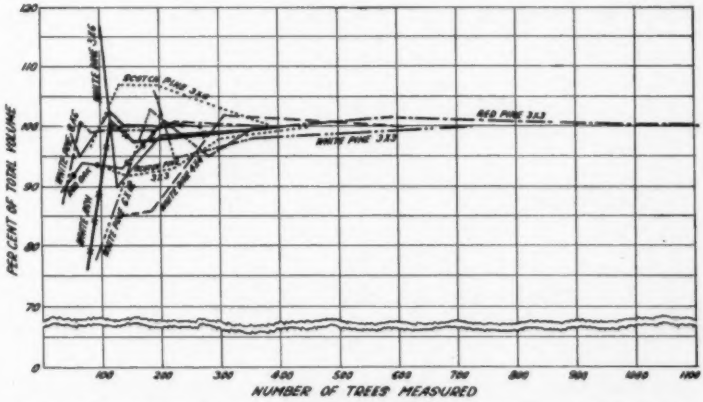
Of the 11 stands studied, 4 must be measured entirely in order to obtain the desired accuracy in diameter, 4 need only a 50 per cent estimate, 2 a 25 per cent estimate, and 1 a 10 per cent estimate. In order to obtain the desired accuracy in height 2 must have a 100 per cent estimate, 1 an 80 per cent, 3 a 50 per cent, 3 a 25 per cent, 1 a 20 per cent, and 1 a 10 per cent estimate.

TABLE 4.—*Percentage of each stand which must be measured in order to obtain the desired accuracy in diameter and height*

| Plot | Spacing of trees | Location | Total number of trees in each stand | Diameter | Height |
|---|---|---|---|---|---|
| | *Feet* | | | *Per cent* | *Per cent* |
| White pine | 4 by 4 | Athens | 612 | 25 | 25 |
| Do | 3 by 6 | Wooster | 404 | 50 | 50 |
| Do | 6 by 6 | Oberlin | 140 | 100 | 100 |
| Do | 6 by 12 | do | 360 | 100 | 50 |
| Do | 3 by 3 | do | 230 | 100 | 50 |
| Do | 3 by 3 | Wooster | 725 | 25 | 25 |
| Scotch pine | 3 by 6 | do | 400 | 50 | 20 |
| Do | 3 by 3 | do | 465 | 50 | 25 |
| Red pine | 3 by 3 | do | 1,155 | 10 | 10 |
| Red oak | 6 by 6 | do | 270 | 50 | 80 |
| White ash | 4 by 4 | do | 400 | 100 | 100 |

From these facts it is apparent that in small, nonuniform stands such as the Oberlin and the white ash stands, less than a 100 per cent estimate will not give the desired accuracy in diameter. In the majority of small but uniform stands, 50 per cent will give accurate results, and in uniform stands of over 500 trees a third or a quarter of the stand will give good results.

The limit of accuracy in height allows for a smaller percentage of the stand to be estimated in some cases than does the limit of accuracy in diameter; in one instance only was it found that more of the


PER CENT OF TOTAL VOLUME
NUMBER OF TREES MEASURED
RED PINE 333
WHITE PINE 333
SCOTCH PINE 333
WHITE ASH 333
WHITE PINE 333
WHITE ASH 333
WHITE PINE 333
WHITE PINE 333
WHITE PINE

nearer the true volume. If this is the case, a smaller percentage of the stand might be measured than that allowed by the probable error of either height or diameter.

Following out this theory, the volumes of the stands studied were computed and also the volumes for the various percentages of each. These were then plotted on a common graph, using the number of trees measured as the abscissa and the percentage of the total true volume as the ordinate. This produced a series of curves (fig. 2), each independent of the others, but all based on the same ordinates.

A glance at these curves brings out the fact that between 200 and 400 trees there is a sharp trend toward a common line. As the number of trees measured increases, the volumes found approach more and more closely the true or 100 per cent volume. Thus, measuring 150 trees in the stands in question gives a volume that is within 9 per cent of the true volume in all cases, 250 trees give a result that is within 5 per cent, and 350 trees give a result within 2 per cent of the true volume.

Allowing an error of ±5 per cent of the true volume, measuring approximately 250 trees in any of the stands in question would give satisfactory results. In uniform stands, which are about the same size as those studied in this problem, the measuring of a representative percentage of the stand which will give about 250 trees should give results which will not vary from the true volume by more than 5 per cent.

TABLE 5.—*Number of trees in each plot, percentage estimate needed, and number of trees this estimate will include*

| Plot | Spacing of trees | Total number of trees | Percentage estimate needed | Number of trees to be measured |
|---|---|---|---|---|
| | *Feet* | | | |
| White pine | 4 by 4 | 612 | 25 | 153 |
| Do | 3 by 6 | 404 | 50 | 202 |
| Do | 6 by 6 | 140 | 100 | 140 |
| Do | 6 by 12 | 360 | 100 | 360 |
| Do | 3 by 3 | 230 | 100 | 230 |
| Do | 3 by 3 | 725 | 25 | 181 |
| Scotch pine | 3 by 6 | 400 | 50 | 200 |
| Do | 3 by 3 | 465 | 50 | 233 |
| Red oak | 6 by 6 | 270 | 80 | 216 |
| White ash | 4 by 4 | 400 | 100 | 400 |
| Red pine | 3 by 3 | 1,155 | 10 | 115 |

Applying this rule, namely, the need of measuring approximately 250 trees in a stand, to Table 5, it is found that in only two stands did the limit of accuracy as determined by the probable error of both diameter and height call for an estimate of more than 250 trees in a stand. This rule of thumb apparently is compatible with the accuracy attained by keeping the probable error of height within ±0.2 foot and the probable error of diameter within ±0.04 inch. In the stands studied, therefore, measuring a percentage of the stand which will include at least 250 trees will give results which are within the limits of accuracy as determined by both volume and probable errors.

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